Optimizing Bioanalysis of Dried Blood Spots

Elham Ataei Alizadeh[1], Georg Rast[1], Chris Cantow [2], Jessica Schiwon[1], Florian Krause[1], Guido De Meyer[3] ,Pieter-Jan Guns[3], Brian Guth[1], Michael Markert[1]

1 Department of Drug Discovery Sciences, General Pharmacology Group, Boehringer Ingelheim Pharma GmbH & Co KG, Germany
2 Department of Drug Discovery Sciences, Bioanalysis Team, Boehringer Ingelheim Pharma GmbH & Co KG, Germany
3 Laboratory of Physiopharmacology, University of Antwerp, Antwerp, Belgium

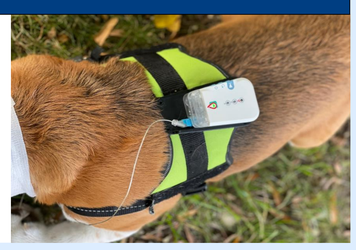
Contact: elham.ataei_alizadeh@boehringer-ingelheim.com

INTRODUCTION

Pharmacokinetic/pharmacodynamic (PK/PD) models are increasingly used in drug development to link drug exposure to pharmacological effects. Multiple blood samples need to be taken concurrently with physiological measurements. The use of automated blood samplers using dried blood spots (DBS) can avoid the hemodynamic changes caused by manual blood sampling from conscious animals. This is particularly advantages for safety pharmacology studies. In spite of the convenience and minimal invasiveness of DBS sampling, it is important to consider potential challenges and differences from conventional plasma measurements. Therefore, it is necessary to optimize bioanalytical protocols and sample preparation to ensure accurate and reliable measurements.

Aims

The objective of this study was to optimize the bioanalytical processing of DBS in comparison to conventional sample preparation methods. The goal was to reduce cardiovascular data variability during safety pharmacology studies and support PK/PD modeling by using a novel blood sampling approach.



METHODS

The study compared dry blood and conventional methods by analyzing compound concentrations. Sample preparation methods, technical approaches, anticoagulant types, and compound types were examined. Beagle dog blood samples spiked with 16 compounds were used.

The study compared the mean percentages of compound concentrations of DBS and plasma from whole blood (WHB) with two anticoagulants.

The study selected compounds of different chemical classes based on the BCS, various pKa values and pH solubility for extraction of compounds based on their solubility.

For extraction, three different liquid solutions (neutral, acidic and basic) were compared. For each, four different concentrations (50 nM,100 nM, 500 nM, 1000 nM) were tested.

It is necessary to transform the DBS results to allow comparison to WHB to predict compound concentrations in vivo. The Cp/Cb ratio is determined by dividing the compound concentration in "Plasma from WHB" by the compound concentration in WHB (figure 1) in order to extrapolate the results from DBS. As a gold standard of reference, plasma from WHB is considered 100% to normalize the results.

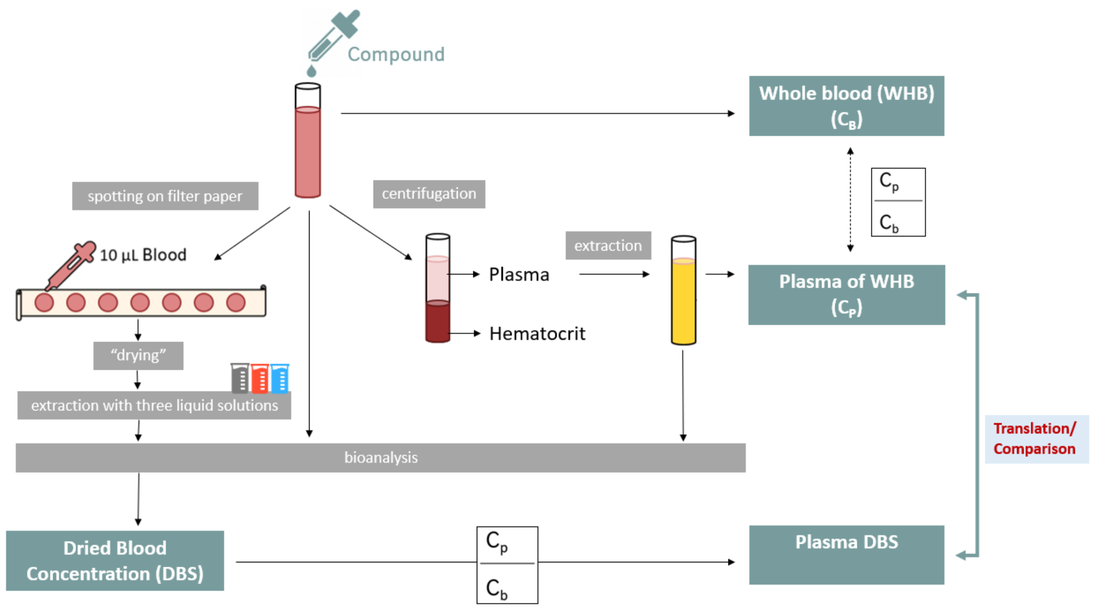


Figure 1. Sample preparation methods for analysis. A) spiked compound into fresh whole blood (WHB), B) compound was spiked into fresh whole blood and incubated for 15 minutes before centrifugation and extraction of the plasma part was carried out, C) compound was spiked into fresh whole blood, and after 15 minutes of incubation, 10 µl of blood were pipetted on paper tape and allowed to dry overnight. "Plasma from WHB" is considered as 100% reference.

RESULTS

There are a number of bioanalysis factors that must be taken into account for an optimal and accurate comparison between DBS and conventional plasma measurements. Based on the bioanalytical analysis, no difference was observed in the compound concentration between the two anticoagulants.

DBS extraction requires a liquid solution based on the pKa of each compound. However, most compounds were extracted from neutral liquid solutions, but using the wrong extraction solution can result in less reliable bioanalysis. The liquid solution should be chosen based on the compound's pKa values. pH also affects compound concentration and stability. Figure 2 shows representative compounds for each BCS cluster.

Despite Cp/Cb exhibiting small biological changes with different compound concentrations in samples, DBS compound concentrations act similarly to normal whole liquid blood. Based on these results, DBS may provide an effective alternative to traditional methods for obtaining blood for various analytical purposes.

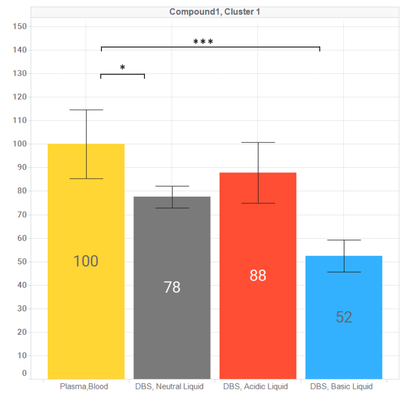
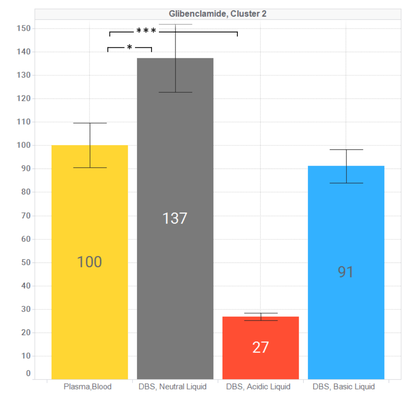
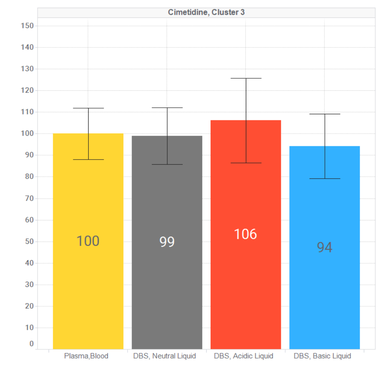


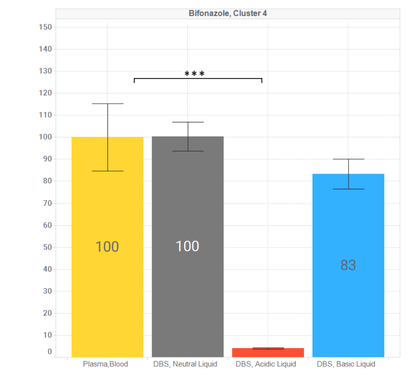
Figure 2. A. Mean (four animals) of compound concentrations of "Plasma from WHB" and DBS in Metoprolol from BCS1.



B. Mean (four animals) of compound concentrations of "Plasma from WHB" and DBS in Glibenclamide from BCS2.



C. Mean (four animals) of compound concentrations of "Plasma from WHB" and DBS in cimetidine from BCS3 .



D. Mean (four animals) of compound concentrations of "Plasma from WHB" and DBS in bifonazole from BCS4..

CONCLUSIONS

DBS sampling has become increasingly common in drug development and safety pharmacology studies due to its advantages, including minimally invasive sampling, small volume requirements, convenient storage and transport. By using wearable automatic blood sampling systems (e.g., Fluispotter), DBS can be collected with minimal disruption to the physiological parameters of test subjects, facilitating PK/PD modelling. The bioanalysis of DBS can provide equivalent results to conventional blood sampling techniques by utilizing the optimized methods and approaches we employed for sample preparation. There is a great deal of potential for wearable automatic blood sampling systems to improve the accuracy and ethical standards of safety pharmacology studies.

doi.org/10.1016/j.vascn.2023.107296.

SPS, September 2023, Brussels, Belgium

Funded by the European Union