In this work we focus on dried blood samples. Seeing the multiple advantages, an increased interest in the use of dried blood samples, with different kinds of applications in various fields, has arisen. On the other hand, dried blood sample analysis is still struggling with some issues. Therefore the objective of this thesis was twofold: first we focused on the possibilities of dried blood samples, with therapeutic drug monitoring (TDM) being the field of interest; second we discussed a major issue related to the use of classical dried blood spots (DBS), being the hematocrit (Hct) effect and applied a new strategy able to cope with this issue.

Alternative sampling strategies can be looked at from two sides: (1) traditional samples (i.e. blood, plasma, serum or urine) collected in an alternative way (e.g. DBS); and (2) alternative samples collected in all kind of ways (e.g. oral fluid or hair). In Chapter A.1., a broader view is provided on distinct alternative sampling strategies used throughout different fields of application, with special attention to the use of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The latter is the technique of choice when considering sensitivity and selectivity. Specific benefits as well as challenges and limitations which have been associated with the different alternative sampling strategies were discussed, this together with recent advances and future trends, which are important for a more routine implementation of these sampling strategies.

One of the best known alternative sampling strategies is DBS sampling. DBS are generally collected as a spot, obtained by a finger or heel prick, on dedicated filter paper. TDM can be considered as one of the fields with an increased interest in the use of dried blood samples (amongst which DBS). The reason is multifaceted: (1) the ease of dried blood sample collection, making home sampling possible; (2) the dried matrix generally induces an improved analyte stability; (3) the compatibility with automated systems, making dried blood sample analysis high-throughput-capable; and (4) the ease of transport and storage. On the other hand, classical DBS analysis also suffers from some issues, with the Hct effect definitely being the most discussed one. Therefore, in an attempt to cope with this issue, different alternative types of devices capable of generating dried blood samples have entered the market, amongst which the volumetric absorptive microsampling (VAMS) devices. VAMS devices preserve all advantages coupled to classical DBS, with the profit of eliminating the Hct effect. Therefore, Chapter A.2. describes the development, validation and application of an ultra-performance liquid chromatography - tandem mass spectrometry (UPLC®-MS/MS)
method for the determination and quantification of four anti-epileptic drugs (AEDs) and one active metabolite, including carbamazepine (CBZ), valproic acid (VPA), phenytoin (PHT), phenobarbital (PB) and carbamazepine-10,11-epoxide (CBZ-E), making use of VAMS devices. These first-generation AEDs, characterized by narrow therapeutic windows, large numbers of drug-drug interactions and severe side-effects, are still frequently used for seizure control in developing countries, where dried blood sampling may be of utmost benefit [5]. Both bioanalytical and VAMS-specific parameters were included within the method validation and overall the pre-set acceptance criteria were met. In this Chapter we also describe how existing external serum quality control (QC) samples can be used as an alternative for usually missing-whole blood QC samples to evaluate the performance of a dried blood-based method.

One of the advantages coupled to DBS analysis is the possibility for automation, making the sampling technique feasible for high-throughput settings. In Chapter A.3., we successfully validated a fully automated DBS method, using a DBS-MS 500 autosampler, online coupled to an LC-MS/MS system, for the determination and quantification of the same set of AEDs as used in Chapter A.2. The DBS-MS 500 system consists of a robotic arm, able to transport cards from the card racks to the different workstations; an optical recognition system used for spot location and information collection; an internal standard (IS) module, which sprays the IS solution onto the DBS cards before extraction; an extraction module, holding a 4 mm clamp head; and a wash station. Method development revealed the importance of thorough optimization of the fully automated extraction procedure, finally resulting in the exclusion of the built-in IS spray. Here again, method validation included the evaluation of both bioanalytical and DBS-specific parameters.

The ease of sample collection and storage in combination with the reduced risk of infection, makes dried blood matrices extremely useful for sampling in remote or resource-limited settings. Therefore, in Chapter A.4. the VAMS- and DBS-based methods described above were used for the analysis of samples originating from children receiving AEDs in Uganda and the Democratic Republic of the Congo. Here, we observed that AED concentrations within the specific therapeutic reference ranges were only present in a relatively low number of patients. However, when comparing these results with the amount of seizures obtained during the last month before sampling, no obvious link could be observed: some patients with a concentration below the therapeutic reference ranges mentioned a seizure decrease, whilst
others with a concentration within the therapeutic range were poorly controlled. The latter illustrates the complexity of TDM of AEDs. Furthermore, an inexplicable underestimation was observed for all analytes when comparing DBS concentrations with VAMS concentrations. Here, a comparative study including VAMS, DBS and whole blood samples could help to address this finding.

The Hct is defined as the volume percentage of blood taken in by red blood cells. The Hct is determined by the amount and the size (volume) of these cells and influenced by different factors, e.g. age, sex, health and nutritional state. Overall, reference ranges lie at approximately 41-50% and 36-44%, for men and women, respectively. However, inter- as well as intra-individual differences exist. When preparing DBS, blood with a higher Hct (e.g. 50%) will spread less over cellulose-based DBS cards, compared to blood with a lower Hct (e.g. 30%), due to differences in the viscosity of the blood. When applying partial-punch analysis, this may impact the validity of the obtained results, since this partial-punch (e.g. 3 mm) originating from a DBS with a higher Hct will contain a larger volume of blood compared to DBS with a lower Hct \cite{2}. Seeing the important impact of the Hct on DBS analysis, Chapter B.1. provides an overview on the recently suggested strategies that may help to cope with the Hct issue. More particularly, a distinction is made between attempts to avoid the Hct issue, strategies to minimize the issue and approaches able to measure or estimate the volume and/or Hct of a DBS.

One of the suggested strategies to avoid the Hct issue is volumetric collection of blood, followed by a whole spot analysis. Recently, different devices, utilizing this strategy, have entered the stage, allowing to maintain the benefits coupled to classical DBS, but eliminating the Hct issue. One example of such recently designed devices is the Capitainer-B device (referred to as microfluidic-DBS or MF-DBS device). In Chapter B.2. we evaluated the potential of this device to effectively eliminate the Hct effect by analyzing 133 left-over patient samples across a wide Hct range (18.8-55%). Furthermore, we investigated whether the amount of blood applied is influencing the performance of the device. To this end, an UPLC®-MS/MS method, making use of caffeine and its metabolite paraxanthine as model compounds, was fully validated, meeting all preset acceptance criteria. It could be concluded that, based on a comparison between analyte concentrations measured in MF-DBS and in corresponding partial punch DBS, the Hct had no impact on the measured concentrations in MF-DBS, this in
contrast to partial punch DBS. Furthermore, addition of different volumes of blood originating from patients with different Hct values at the inlet of the device, demonstrated that the amount of blood added has no influence on the device performance, this independently from a patient’s Hct.

Conclusively, in the field of alternative sampling strategies an enormous progress has been made during the past few years. However, despite the numerous new developments, there are still some limitations which need to be tackled, e.g. the (relatively) high cost associated with most of the newly developed strategies. Furthermore, an evaluation of several of the new strategies by using capillary blood, applied directly from a fingertip under real-life circumstances, is still often missing, which is essential for the evaluation of their user-friendliness and robustness in real practice. The latter is very important for the acceptance of the different new developments as a reliable alternative for whole blood analysis. Finally, one should also bear in mind that there will always be some limitations coupled to dried blood based-methods, e.g. in some cases the analytical result is urgent, excluding the possibility to wait until the sample has dried. In these circumstances, the use of the classical, venous sampling technique may be necessary. However, an -already proven- alternative is the use of wet microsamples. On the other hand, the use of dried blood samples can definitely be encouraged when wet sampling is not possible, e.g. in remote areas or for home monitoring.
References


