Blood Hematocrit Still a Critical Parameter in Bioanalysis

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Editorial

Recent developments in bioanalytical techniques including both sample preparation and analysis have reassured the decrease in blood sample size, a fact that is of utmost importance in cases when the available sample size is limited as for example in neonates, in postmortem samples or other populations with specific limitations e.g. anemic people, patients, elders etc. Moreover minimizing sample volume also complies with the twelve principles of green analytical chemistry.

Drugs and their metabolites can be monitored in samples of reduced volume to produce valuable and reliable results in pharmacokinetic studies, toxicokinetic studies, disease diagnoses, disease prognosis in metabolomics studies, therapeutic drug monitoring, forensics etc. Therefore microextraction techniques or micro sampling techniques have been introduced.

Dried blood spot sampling is an effective approach to this direction. Implementing dried blood microsamples in the bioanalytical workflow renders sample collection, transport, storage and processing much simpler and user friendly. It also enables the sampling in the patient’s home, if necessary.

However it is known that blood hematocrit (Hct) can play a determining role in those analyses as for example in dried blood spot sampling by affecting the diameter of the derived Dried Blood Spots (DBS) sample. Hematocrit influences not only the homogeneity of sample but drug distribution as well. This is because the viscosity of blood samples which is directly related to their Hct values, may lead to non-homogenous spots since and samples with higher Hct values yield spots with smaller diameter and vice versa. Moreover taking a fixed-sized sub-punch from a high Hct DBS will contain a higher amount of blood (and consequently of analyte) than a punch taken from a low Hct DBS and this leads to a Hct-dependent assay bias. Not to mention that high Hct levels may influence the nalytes’ recovery from DBS [1-10].

As it is well known the term Hematocrit refers to the fraction of blood volume that is consisted of red blood cells (erythrocytes), which comprise one of the major constituents of whole blood besides the plasma. Although plasma and serum are the preferred samples for most analytical procedures, the analysis of whole blood samples is often required as for example in post mortem cases or in cases where the shipment of the sample can be simplified and safer. In these cases the evaluation of Hct’s effect on whole blood samples analysis should be taken into consideration.

To overcome this negative effect, the analysis of whole spot is recommended. However, this approach requires the accurate and precise application of a fixed volume of blood onto the filter paper using suitable pipettes or microcapillaries. But this approach is not feasible when sampling is to be carried out by non-qualified personnel as for example in patient’s home.

Alternative ways to overcome the hematocrit issue involve “Volumetric absorptive microsampling” (VAMS), which is a simple technique for collecting and quantitative analysis of dried blood samples. It enables the collection of an accurate blood volume (approximately 10 μL), which is independent of the HCT of the blood, therefore no specialized devices, such as pipettes or capillaries are required. This device consists of a plastic handler with an absorbent polymeric tip, which is attached. This tip when dipped into blood, wicks up an accurate volume of blood.

Microfluidic devices consisting of a foldable support system that holds a DBS card on one side and a microfluidic plate with sized capillaries on the other have been also introduced. Other approaches such as the application of microextraction techniques such as Solid-phase microextraction (SPME) or liquid-liquid microextraction are also applied but they have been also reported to be affected by hematocrit. These problems might be overcome by using appropriate internal standards. However various analytes may be affected by Hct in a different way, therefore erythrocytes levels should be always properly considered when analyzing whole blood samples with microextraction techniques among other operation experimental conditions in the bioanalytical laboratory [11-17].

References


