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Dried blood spots analysis with mass spectrometry: Potentials and pitfalls in therapeutic drug monitoring

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ABSTRACT

Therapeutic drug monitoring (TDM) relays in the availability of specialized laboratory assays, usually available in reference centers that are not accessible to all patients. In this context, there is a growing interest in the use of dried blood spot (DBS) sampling, usually obtained from finger pricks, which allows simple and cost-effective logistics in many settings, particularly in Developing Countries. The use of DBS assays to estimate plasma concentrations is highly dependent on the hematocrit of the blood, as well as the particular characteristics of the measured analyte. DBS assays require specific validation assays, most of them are related to hematocrit effects. In the present manuscript, the application of mass spectrometric assays for determination of drugs for TDM purposes in the last ten years is reviewed, as well as the particular validation assays for new DBS methods.

1. Introduction

Therapeutic drug monitoring (TDM) relays in the availability of sophisticated laboratory assays in order to be performed properly. Usually these assays are available in specialized reference centers, not accessible to all patients. In this context, there is a growing interest in the use of dried blood spot (DBS) sampling, usually obtained from finger pricks, which allows simple and cost effective logistics in many settings, particularly in Developing Countries. Other additional advantages of DBS for TDM, reviewed by Edelbroek et al. (2009) [1] and Wilhem et al. (2014) [2], include minimally invasive sampling, high analyte stability and the possibility of self-sampling by patients.

However, DBS sampling is not free of drawbacks. Self-sampling could be associated to contaminations and samples with unacceptable quality. Clinical use of DBS data for TDM requires extensive clinical validation is once capillary blood from finger pricks could present different concentrations from venous blood. Additionally, varying hematocrits affect the volume of blood in a spot with fixed diameter obtained from a supporting matrix the blood-to-plasma partition of the measured analyte. Moreover, the small amount of sample available for testing, usually in the range of 5 to 50 μL, is demanding to the testing laboratory. This latter issue is usually assessed by using analytical methods based on mass spectrometry, either gas chromatography–mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS/MS), which associates the high sensitivity and specificity required for TDM purposes, particularly for the small DBS samples [3]. The availability of GC-MS and LC-MS/MS instruments in clinical laboratories greatly improved the analysis of DBS samples in the current decade.

The aim of this manuscript is to review the application of mass spectrometric analytical methods for DBS testing in the context of TDM, discussing the clinical application of this alternative sampling strategy and the specific assay development and validation issues to be addressed in order to implement DBS assays, with a focus in published applications in the last 10 years.

2. Use of DBS to estimate plasma concentrations

The interpretation of drug concentration measurements in the context of TDM usually is based on reference ranges established in plasma or serum samples. As DBS are essentially measurements in whole blood, there is a need to convert the information obtained in this matrix to plasma levels. The hematocrit (Hct) of the sampled blood has a major influence in this process for two major reasons: 1) the viscosity of blood affects the amount of sample present in a matrix punch of fixed size, in a way that is dependent of the particular matrix used for spotting the blood, and 2) the proportion of red blood cells and plasma in the sample modifies the relative concentration of the drug on these blood compartments.

The majority of published applications of DBS sampling for TDM had evaluated Hct ranges where accuracy and precision of the assays were acceptable and made no correction for the varying amount of sample due to different Hct values. However, Vu et al. (2010) [4], using several different cellulose matrices for DBS, estimated the resulting volume of blood in the spot in Hct values of 0.20, 0.35 and 0.50 for moxifloxacin determination. For the three Hct values, blood spot areas (A_{DBS}) obtained

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after spotting volumes in the range of 10 to 60 μL were measured from digital images, using a specific software, and the estimated volume in the spot (V_{est}) was calculated with equation $V_{est} = (\pi \cdot r^2 \cdot V_b) / A_{DBS}$, being spot radius (r) fixed at 4 mm. This approach was based on the assumption of a linear relation between applied volume of blood and DBS area. Additionally, regression lines correlating V_{est} (y) and Hct (x), normalized to 0.35 (the Hct used for preparation of calibration samples — Hct_{cal}) were obtained, being the intercept denominated as V_{std} and the angular coefficient denominated b. A corrected concentration of the analyte in the DBS was calculated using the equation $C_{corrected} = C_{measured} \cdot (V_{std} / V_{est})$ or using the regression coefficients, $C_{corrected} = C_{measured} \cdot \{V_{std} / [V_{std} + b\ (Hct\text{-}Hct_{cal})]\}$. Using Whatman™ 31 ET CHR paper as DBS matrix, the difference between uncorrected moxifloxacin concentrations between lowest (0.20) and highest (0.5) Hct was about 40%, falling to below 15% after the correction procedure described above.

Besides variations in the amount of blood present in a DBS of constant size, the determination of the concentration of a drug in plasma from a DBS measurement also requires knowledge of the its partition between the cellular and the water compartments of blood. The ratio between concentrations of a drug measured in blood and plasma is dependent of the unbound fraction in plasma (f_u) and the erythrocyte-to-plasma concentration ratio (ρ), as well as the Hct [5]. According to Rowland and Emmons [5], the major concern for estimating plasma concentrations from whole blood measurements for drugs with a blood-to-plasma concentration ratio close to the lower limit of 0.55, which indicates almost complete amount in plasma, is the variability in f_u. In the other hand, for drugs with larger values of the blood-to-plasma concentration ratio, particularly higher than 2, variability in ρ becomes the critical factor. However, for drugs showing little variability in f_u and ρ under clinical conditions, there is little concern in the use of DBS as an alternative to plasma.

An estimated plasma concentration can be calculated using the equation $C_{plasma} = C_{blood} / [(1\text{-Hct}) + Hct \cdot f_u \cdot \rho]$ [5]. When f_u is constant, an alternative approach is based on the knowledge of the fraction of the drug in plasma (f_p), which can be established in an in vitro experiment as previously described [6], and is calculated as $f_p = (C_{blood} / C_{plasma}) \cdot (1\text{-Hct})$. Once f_p is known, the plasma concentration can be calculated using the simplified formula $C_{plasma} = [C_{blood} / (1\text{-Hct})] \cdot f_p$ [6–8].

Moreover, as DBS is usually based on blood obtained from finger pricks and is composed from arterial capillary blood and some amount of interstitial fluid, drug concentrations can potentially be different from venous blood. As an example, Ashley et al. [9] found concentrations of piperaquine about 1.7 times higher in capillary when compared to venous blood. As these differences are dependent of the characteristics of particular drugs, case-to-case evaluation is necessary during clinical validation. The use of linear regression correlating plasma and DBS concentrations from clinical data in the validated range of Hct values could potentially account for overall differences in drug concentrations in both matrices [8].

Based on the above, it is obvious that knowledge of hematocrit is critical to the translation of DBS analysis to interpretable plasma levels. The most straightforward approach currently available to determine the Hct of a DBS was proposed by Capiau et al. (2013) [10], and is based on the measurement of the concentration of potassium in a DBS punch. The authors found a linear correlation between potassium concentrations and Hct in the range of 0.19 to 0.63, with acceptable accuracy and precision. Moreover, concentrations of potassium in DBS were stable up to 55 days at room temperature. This approach can be used to calculate plasma concentrations using the described equations or to evaluate if a particular sample has a Hct within the validated range of a certain method.

Even considering that a stable relation between concentrations measured in DBS and plasma could be estimated by validation and in vitro experiments, a clinical validation study with patient samples is a mandatory step before implementation of a DBS assay in routine.

3. Quality assurance and validation of DBS assays

An adequate quality control is essential to ensure meaningful data in TDM testing. Several pre-analytical, analytical and post-analytical variables influence the DBS analysis and must be taken into account during the development and validation of a new DBS assay, as discussed below.

3.1. Choice of matrix

During method development, testing for the most appropriate type of matrix can be an efficient strategy to optimize the DBS analysis. The most common matrix for DBS are cellulose-based papers. Papers are mostly differentiated by its composition, thickness and resistance to spreadability of blood. These characteristics may give rise to differences in extraction recovery, matrix effects, analyte stability and chromatographic, Hct and volume effects [11].

There are two main types of commercially available paper cards suitable for DBS: chemically untreated and treated papers. The untreated are the most commonly used, particularly the pure cellulose Whatman 903® and Ahlstrom 226®, which are registered by the US Food and Drug Administration (USFDA). Novel substrate materials, allegedly less subject to Hct effect on spot size and analyte recovery are under research, such as the Agilent Bond Elut DMS® card, with promising results [2]. The chemically treated group of matrices consists of cellulose papers treated with different proprietary chemicals intending to lyse cells, inactivate pathogens, and denature enzymes and other proteins. Among them, Whatman FTA®, FTA Elute®, FTA DMPK-A® and FTA DMPK-B® are the most used in this group. Alternatively, untreated paper can be impregnated with chemicals in order to improve stability of some analytes [11].

3.2. Collection procedure

DBS sample collection procedures must follow uniform procedures to minimize the potential effects of pre-analytical errors, such as overlapping or smeared spots. Peck et al. (2009) evaluated the variation of blood volumes and geometries of 422 DBS samples obtained from 138 patients. The study showed non-idealities from blood spot collection, including low blood volumes (2 to 72 μL; mean: 25 ± 13 μL), multiple-drop applications, and aberrant sample geometries not consistent with single-drop applications, indicating the need for continuing education of bloodspot collectors [12].

Contamination is another concern for DBS sampling, which can lead to inaccurate determinations. It can result from the use of topic anesthetic creams, disinfectants and handling of the drug prior to collection. In this regard, the European Bioanalytical Forum (EBF) proposed the concept of good blood-spotting practices (GBSP) [13,14].

An outline for DBS collection procedure is listed below [11,15,16]:

1. Prior to the collection, any contact with the target site of the matrix card must be avoided.
2. If the participant's hands are cold, massaging or warming the collection site before pricking can stimulate local blood flow.
3. Clean puncture site with 70% isopropyl alcohol.
4. Use a sterile, single-use lancet to prick the finger just off the center of the tip of the middle or ring finger (lancets with blades produce better blood flow than needles).
5. Wipe the first blood drop away with a sterile gauze pad to remove the tissue fluid from the sample.
6. Carefully position the collection paper below the finger and allow the drop to fall of its own weight. Up to five drops of blood (average of 50 μL per drop) are applied to the DBS matrix. For a better blood flow, gently milk the hand starting at the wrist and work down to the base of the finger to produce blood flow, avoiding to squeeze the finger. The donor's finger should never touch the DBS matrix.