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EXPERT REVIEW

Dried Blood Spot Technique Applied in Therapeutic Drug Monitoring of Anticancer Drugs: a Review on Conversion Methods to Correlate Plasma and Dried Blood Spot Concentrations

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ABSTRACT

Background Anticancer drugs are notoriously characterized by a low therapeutic index, the introduction of therapeutic drug monitoring (TDM) in oncologic clinical practice could therefore be fundamental to improve treatment efficacy. In this context, an attractive technique to overcome the conventional venous sampling limits and simplify TDM application is represented by dried blood spot (DBS). Despite the significant progress made in bioanalysis exploiting DBS, there is still the need to tackle some challenges that limit the application of this technology: one of the main issues is the comparison of drug concentrations obtained from DBS with those obtained from reference matrix (e.g., plasma). In fact, the use of DBS assays to estimate plasma concentrations is highly dependent on the chemical-physical characteristics of the measured analyte, in particular on how these properties determine the drug partition in whole blood.

Methods In the present review, we introduce a critical investigation of the DBS-to-plasma concentration conversion methods proposed in the last ten years and applied to quantitative bioanalysis of anticancer drugs in DBS matrix. To prove the concordance between DBS and plasma concentration, the results of statistical tests applied and the presence or absence of trends or biases were also considered.

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INTRODUCTION

Many anticancer drugs are characterized by a low therapeutic index. Moreover, they exhibit large pharmacokinetic (PK) inter-individual variability following standard dosage regimens (i.e., from about 1 up to 26-fold, for targeted therapies) (1). Drug concentration measurement in blood offers the unique opportunity to know whether drug levels are adequate or if a dose adjustment is needed to achieve the effective drug plasma concentration with acceptable toxicities (2). This practice is defined as Therapeutic Drug Monitoring (TDM) and allows to personalize the dosage, which is imperative in the case of anticancer treatments (3). Despite this shared opinion, the application of TDM in clinical practice is not widespread due to several limitations that hamper most of the published TDM methodologies to be translated in a cost-effective and patient-friendly routine diagnostic tool. Among these limitations sample collection remains one of the main TDM constraints due to the requirement of specialized personnel to perform venous sampling and sample processing. In this scenario, dried blood spot (DBS) could represent a useful strategy to overcome sample collection limitations.

It is a microsampling technique, in which a capillary blood drop, derived from a simple finger-prick, is collected on a filter paper. Its first use is dated to 1963 by Guthrie and Susi (4). Nowadays, due to the higher affordability of sensitive techniques (such as LC-MS/MS), DBS is becoming an alternative matrix for drug exposure measurement in many fields. More recently, there is a growing interest in DBS application also for TDM of anticancer drugs. In fact, DBS collection through finger prick would improve patients' compliance and simplify pre-analytical steps. In addition, the drying process enhances

the stability of most compounds, enabling more cost-effective transport and storage (5). Finally, DBS may be performed directly by the patient him/herself at home and sent to the hospital, allowing laboratory results to be available before the patient visits a clinician for the routine follow-up.

Despite the significant advances in bioanalysis exploiting DBS, there are still some challenges that limit its application. In fact, analytical methods based on DBS require an extensive analytical validation to evaluate the impact of additional parameters in the analysis, such as hematocrit (Hct) value, spot size, DBS homogeneity, the difference between capillary and venous drug concentration as reported in several specific guidelines (6,7). Moreover, DBS-based analytical methods require also a clinical validation, that is the establishment of the correlation between DBS and plasma concentrations. In fact, blood, rather than plasma, concentration is the direct result of DBS analysis, instead the interpretation of drug concentrations in TDM context is usually based on reference ranges established in plasma or serum. Thus, a conversion of DBS concentrations is needed to correlate them with plasma measurements. This should ideally be independent from patient, sample collection time, and administered dose.

To understand the feasibility of DBS technique in TDM context, the chemical-physical properties of the drug and their impact in blood distribution (such as the blood to plasma partition and the influence of Hct) have to be considered. Consequently, each drug requires an extensive study to find a precise approach to normalize DBS concentrations to those measured in plasma.

In this scenario, the aim of the present review is to perform a critical investigation of the DBS-to-plasma concentration conversion methods applied to quantitative bioanalysis of anticancer drugs in DBS matrix through the collection and examination of the literature from November 2008 until May 2020. To meet this objective, the results of statistical tests applied in bioanalytical methods to demonstrate the concordance between DBS and plasma concentrations, as well as the presence or absence of trends or biases, were considered.

MATERIALS AND METHODS

The research was limited to papers published from November 2008 until May 2020. The consultation was performed with Pubmed® (NCBI) database in three different modalities considering only English language articles:

1. using MeSH terminology associated with the Medline database. The research was conducted using (drug monitoring) AND (dried blood spot testing), obtaining 251 papers;
2. exploiting "dried blood spot and therapeutic drug monitoring" applying also a "Cancer" filter, which led to obtain 66 results;
3. analysing all 25 papers considered by Sulochana *et al.* in their review (8).

After a careful examination, only 39 articles (21, 6 and 12 for the first, second and third criteria, respectively) were selected for this review: papers related to DBS analysis of drug not belonging to anticancer family were excluded.

Afterward, another screening was performed: 14 (5, 2, 7 for the first, second and third criteria, respectively) (9–22) were excluded because they did not report any correlation study between DBS and plasma concentrations, while 4 articles (23–26) were excluded as the analyses were performed on non-human matrices (e.g., mice or rats).

The papers actually taken into consideration for this review were therefore 21, as shown in Fig. 1.

The following data were extracted from the selected studies: analytes considered, matrix used for the analysis (DBS, plasma, whole blood, etc.), type of detection (MS/MS, UV-vis, fluorescence), method of correlation between DBS and plasma/whole blood concentrations, number of patients and sample size, as reported in Table I.

CF: correction factor; DBS: Dried Blood Spot; DBS (FP): Dried Blood Spot from finger prick; DBS (V): Dried Blood Spot venous; DBS (FP + V): Dried Blood Spot from finger prick and venous; DC: direct correlation; DPS: Dried Plasma Spot; FLU: fluorescence detector; F_p: blood-to-plasma drug concentration ratio; f_u: unbound drug fraction in plasma; Hct: hematocrit; $K_{BC/pla}$: blood-cell to plasma partition coefficient; MS/MS: tandem mass spectrometry; PLA: plasma; SA: statistical analysis; VAMS (FP): volumetric absorptive microsampling from finger prick; WB: whole blood.

CLINICAL VALIDATION STUDY

Clinical validation is necessary to evaluate the agreement between the two methods (DBS and plasma/serum) and to establish a correlation between DBS and plasma or serum (reference matrix) concentrations. Only after a successful clinical validation study, DBS can replace the standard venous blood sampling and can, therefore, be interchangeably used instead of serum or plasma analysis.

To compare two measurements (i.e., two analytical methods), according to the Guideline provided by the IATDMCT society (7), regression analysis should be performed to evaluate the correlation, followed by an agreement and bias estimation test. Due to some inherent variability of the reference and the DBS methods, either Passing-Bablok or weighted Deming regression should be used to perform the above-mentioned comparison rather than standard linear regression. To assess the agreement between the methods and to estimate possible biases, a Bland-Altman difference plot is also useful(48).

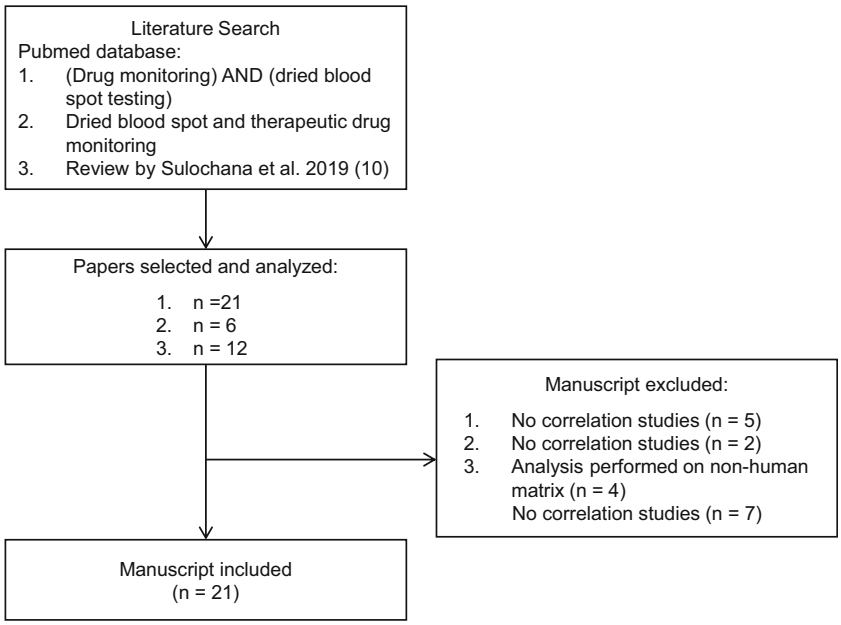

Fig. 1 Flow diagram of literature search and selection of included studies.

Table I Selected Manuscripts

Analyte(s)	Matrix	Detection	Correlation	Patients number	Sample size	Ref
everolimus	DBS (FP), WB	MS/MS	DC	1	5	(27)
	DBS (FP + V), WB	MS/MS	DC	20	20	(28)
	VAMS (FP)	MS/MS	DC	10	25	(29)
	DBS (FP), WB	MS/MS	DC	60	83**	(30)
busulfan	DBS (V), PLA	MS/MS	DC	10	30	(31)
	DBS (V), DPS	MS/MS	DC	15	153	(32)
etoposide	DBS, PLA	FLU	Hct	28	28	(33)
tamoxifen, endoxifen	DBS (FP), PLA	MS/MS	Hct + F_p	44	44	(34)
tamoxifen, N-desmethyltamoxifen, 4-hydroxytamoxifen, endoxifen	DBS (FP), PLA	MS/MS	Hct + CF	91	91	(35)
Irinotecan, SN38	DBS (FP), PLA	FLU	Hct + F_p; SA; CF	19	38	(36)
vemurafenib	DBS (FP), PLA	MS/MS	Hct + $K_{BC/pla}$; SA	8	43	(37)
paditaxel	DBS (FP + V), PLA	MS/MS	Hct + F_p; SA; CF	34	34	(38)
docetaxel	DBS (FP + V), PLA	MS/MS	Hct + F_p; CF	31	31	(39)
imatinib	DBS (V), PLA	MS/MS	Hct	23	23*	(40)
	DBS (FP), PLA	MS/MS	Hct + F_p; CF	50	50	(41)
imatinib, norimatinib	DBS (FP + V), PLA	MS/MS	Hct; CF	26	67	(42)
nilotinib	DBS (V), PLA	MS/MS	Hct	23	23*	(40)
	DBS (FP), PLA	MS/MS	Hct + F_p + f_u; SA	20	40	(43)
dasatinib	DBS (V), PLA	MS/MS	Hct	23	23*	(40)
pazopanib	DBS (FP + V), PLA	MS/MS	Hct	12	95	(44)
	DBS (FP), PLA	MS/MS	Hct + F_p; SA	30	221	(45)
gefitinib	DBS (FP), PLA	MS/MS	DC	10	10	(46)
radotinib	DBS (FP), PLA	MS/MS	Hct; SA	45	45	(47)

*value obtained for imatinib, dasatinib and nilotinib samples together;

**value obtained for everolimus and sirolimus samples together;

The sequential steps generally described in the published methods are the following:

1. Checking the linear correlation between the obtained DBS concentrations and the measured plasma values (references).
2. If there is no linear correlation between DBS and plasma concentrations, applying one or more DBS to plasma concentrations conversion methods based on both drug characteristics and empirical data to obtain the estimated plasma concentration (EC_{pla}) from DBS measurements.
3. Checking the agreement between the EC_{pla} and the actual plasma concentration through statistical analyses (i.e., Passing–Bablok or weighted Deming regression and Bland-Altman plot). Additionally (or alternatively), it is possible to check the agreement with Food and Drug Administration (FDA)/European Medicines Agency (EMA) guideline requirements: at least two-thirds (67%) of the sample results obtained with the new method (DBS) should be within 20% of the results obtained with the standard assay (plasma/serum) (49,50). Alternatively, authors can establish a *priori* the acceptance criteria.

The Clinical and Laboratory Standards Institute (CLSI) guideline states that the sample size necessary for a clinical validation should be of at least 40 patients' samples, covering the entire measuring interval and/or inter-individual variability (52). These samples should be used to evaluate the correlation between DBS and reference concentrations and to identify the DBS-to-reference values conversion method. Furthermore, ideally 40 more patients' samples should be used to validate the conversion method selected. Thus, a total of 80 patients' samples should be collected for a proper validation, which is indeed a large sample size considering the difficulty of collecting a wide number patients' samples, especially in the case of recently approved drugs and rare diseases. In fact, as reported in Table I, most of the published methods were validated on a lower sample size and rarely the authors tested the DBS to plasma concentrations conversion method on an independent set of patients' samples. In most cases, several samples are collected from few patients. Consequently, the results of statistical tests and the confidence interval have to be carefully evaluated since most of the data could be not independent.

PHARMACOKINETIC CONSIDERATIONS IN THE USE OF DBS FOR TDM

Reference drug range or target used in TDM are traditionally defined for plasma (or serum) matrix. Considering that DBS measurements represent drug concentration in whole blood, there is the necessity to figure out both the drug distribution between plasma and blood cells and drug binding to plasma proteins to properly understand the correlation between DBS and plasma/serum concentrations. In fact, drugs are distributed in whole blood between plasma and blood cells compartments and can be bound or unbound to both plasma proteins and cells constituents. The equilibrium that describes this situation is reported in Fig. 2. A drug, in plasma, exists in two forms: bound to plasma proteins or unbound. Only the unbound drug is available to enter blood cells, where it can bind to cell constituents according to its chemical-physical characteristics. As reported by Rowland and Emmons (53,54) drug concentrations in plasma (C_{pla}) and in whole blood (C_{WB}, interchangeable with C_{DBS}) can be defined as follows:

$$C_{pla} = \frac{C^u_{pla}}{f_u} \tag{1}$$

$$C_{WB} = \left[\frac{1-Hct}{f_u} + Hct*\rho\right]*C^u_{pla} \tag{2}$$

where C^u_{pla} is the unbound plasma concentration of the drug, f_u is the unbound drug fraction in plasma and ρ is the blood cells-to-unbound plasma concentration ratio (that measures the affinity of the drug to blood cells). From these two equations, a first relationship between C_{pla} and C_{WB} (or C_{DBS}) can be defined by the following formula:

$$C_{pla} = \frac{C_{WB}}{(1-Hct) + Hct*\rho*f_u} \tag{3}$$

From Eq. (3), it is evident that, besides the partitioning process, volumes of both plasma and blood cells, and thus Hct value, can significantly influence drug concentration in the two compartments. Moreover, Hct can have a direct influence in accuracy and precision of the analytical assay. In fact, variation in Hct values, and thus in blood viscosity, affects blood spreading in the DBS paper and it may also have an impact on extraction efficiency and, thus, on drug recovery (55,56). These aspects need to be evaluated during the

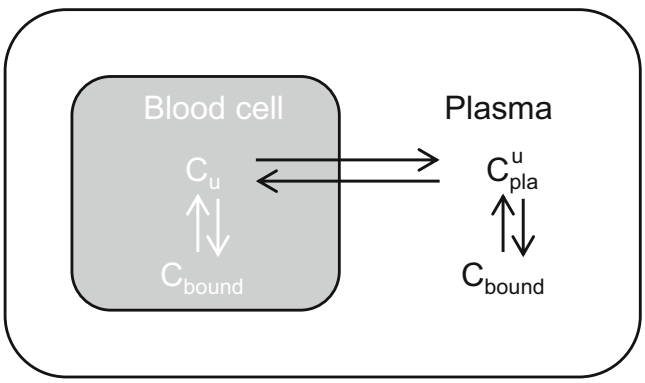

Fig. 2 Schematic description of drug's equilibrium partitioning into the whole blood.

analytical validation and are detailed in the specific guidelines (7). Normally, Hct ranges from 0.41 to 0.51 L/L in men and from 0.37 to 0.47 L/L in women (57), nevertheless, it can be significantly different in specific populations, such as oncologic patients.

Equation (3) can be expressed using the blood cell-to-plasma partition coefficient ($K_{BC/pla}$) which is the concentration ratio between plasma and blood cells compartments (C_{BC}), as indicated in Eq. (4):

$$K_{BC/pla} = \frac{C_{BC}}{C_{pla}} \tag{4}$$

$K_{BC/pla}$ is subjected to drug chemical-physical characteristics, like drug cell permeability and binding to plasma proteins. This latter factor affects f_u, since the higher the protein binding, the lower the f_u of the drug and thus the $K_{BC/pla}$. This direct relationship between f_u and $K_{BC/pla}$ can be noticed if Eq. (1) is introduced in Eq. (4), then $K_{BC/pla}$ can be expressed as follows:

$$K_{BC/pla} = \frac{C_{BC}}{C_{pla}^{u}} * f_u = \rho * f_u \tag{5}$$

Equation (5) can be used to express Eq. (3) as reported below:

$$C_{pla} = \frac{C_{WB}}{(1 - Hct) + Hct * K_{BC/pla}} \tag{6}$$

This equation represents one of the applied DBS-to-plasma conversion methods reported in the literature, as discussed in section 5.1.

Another important parameter is the blood-to-plasma drug concentration ratio (F_p). F_p could be expressed by the following Eq. (7):

$$F_p = \frac{C_{WB}}{C_{pla}} = 1 + Hct * (f_u * \rho - 1) \tag{7}$$

Considering $K_{BC/pla}$ and F_p, different scenarios can be applied to evaluate the DBS measurement. Firstly, if we assume that the drug is equally partitioned into blood cells and plasma, we obtain the following relationships:

$$K_{BC/pla} = \frac{C_{BC}}{C_{pla}} = 1 \tag{8}$$

$$F_p = \frac{C_{WB}}{C_{pla}} = 1 \tag{9}$$

Thus, the concentration in blood (or DBS) is equivalent to the concentration in plasma, facilitating the conversion between DBS/blood and plasma data (i.e., direct correlation).

On the contrary, if we assume that the drug is isolated in plasma and $K_{BC/pla}$ is equal or near to 0, and consequently F_p is about 0.55–0.60, the correlation between plasma and DBS/blood concentrations just depends on Hct: in this case, blood cells only dilute drug concentration. Therefore, Eq. (6) can be simplified as follows:

$$C_{pla} = \frac{C_{WB}}{1 - Hct} \tag{10}$$

In this case, using C_{DBS} instead of C_{WB} in Eq. (10), it is possible to calculate the expected concentration in plasma, as reported in some studies described in section 5.2.

The last scenario occurs when F_p or $K_{BC/pla}$ are greater than 1. In this case, the drug is mainly sequestrated by red blood cells and, according to Eq. (7), it is useful to understand which parameter (f_u or ρ) has a more significant influence.

NORMALIZATION APPROACHES APPLIED TO DBS METHODS FOR ANTICANCER DRUGS

A specific analysis of the different DBS-to-plasma concentration conversion methods reported so far in the literature has been conducted. In particular, according to the researches carried out, 3 types of used normalizations can be outlined: a normalization that considers patient's Hct and the influence of blood-to-plasma partitioning, a simplified normalization only based on patient's Hct value for drugs that do not enter into blood cells compartment, and a conversion based on empirical data (i.e., based on DBS and plasma concentrations found in patients' samples). In this latter case, the conversion can be performed using two different approaches: the use of a correction factor (CF) or the normalization through the application of linear regression analysis. Finally, for drugs equally distributed between blood cells and plasma, no DBS-to-plasma conversion method needs to be applied and a direct correlation can be performed. In the following paragraphs, these approaches are described in detail.

Normalization Based on Blood-to-Plasma Partitioning and Hct

The first case considered is the general situation in which both Hct and blood-to-plasma partitioning need to be considered to properly correlate DBS concentrations (C_{DBS}) with the reference C_{pla}. The equations describing this condition are (3,6) (section 4), where C_{WB} is replaced by C_{DBS} and C_{pla} is substituted with the estimated plasma concentration (EC_{pla}):

$$EC_{pla} = \frac{C_{DBS}}{(1 - Hct) + Hct * \rho * f_u} \tag{11}$$

$$EC_{pla} = \frac{C_{DBS}}{(1 - Hct) + K_{BC/pla} * Hct} \tag{12}$$

Equation (11) was applied by Boons *et al.* (43) in their LC-MS/MS method for the quantification of nilotinib in finger-prick DBS samples collected from patients affected by chronic myeloid leukemia (CML). EC_{pla} was predicted from C_{DBS} using three methods: application of Eq. (11) with either (1) individual or (2) mean Hct value and (3) application of the bias between plasma and DBS concentrations (section 5.4). The analysis was conducted on 40 paired DBS and plasma samples collected from 20 patients in treatment with nilotinib. In order to apply Eq. (11), the authors considered the following data from the literature (58): nilotinib plasma protein binding is approximately 98%, thus f_u was 0.02 and the blood-to-plasma ratio of nilotinib is 0.71, thus $\rho = Hct*0.71$. With both methods (1) and (2) (where the mean Hct from the study population was 0.41 ± 0.05 L/L) EC_{pla} was highly correlated with actual plasma concentration (Table II).

An example of Eq. (12) application, was represented by the LC-MS/MS method for tamoxifen and (*Z*)-endoxifen quantification in finger-prick DBS, proposed by Jager *et al.* (34). The comparison between calculated and actual serum concentration (reference value) to evaluate DBS analysis feasibility was conducted on 44 samples. To apply Eq. (12), a fixed value of Hct was used, corresponding to the mean Hct value for the target population (0.41 L/L) while $K_{BC/pla}$ was determined using fresh human whole blood (Hct 0.41 L/L) spiked with the analytes at low, medium and high concentrations. Plasma was then separated from blood cells, analyzed and C_{BC} was calculated using the following equations:

$$C_{WB} = \frac{C_{pla}*V_{pla} + C_{BC}*V_{BC}}{V_{WB}} \quad (13)$$

$$V_{BC} = Hct*V_{WB} \quad (14)$$

$$C_{BC} = \frac{C_{WB}*V_{WB} - C_{pla}*V_{pla}}{Hct*V_{WB}} \quad (15)$$

Where V is the volume of plasma (V_{pla}), blood cells (V_{BC}) and whole blood (V_{WB}). Thus, $K_{BC/pla}$ was determined by dividing the calculated C_{BC} by the measured C_{pla}: it was 0.461 for tamoxifen and 0.179 for (*Z*)-endoxifen. With Deming regression analysis, the slope and intercept were not significantly different from 1 and 0, respectively, for both analytes the Bland-Altman difference plot showed a small bias for the estimated serum concentration (Table II).

Similarly to Jager *et al.*, Eq. (12) was applied to calculate the EC_{pla} of vemurafenib (37), an oral tyrosine kinase inhibitor (TKI) approved for the treatment of BRAF V600 mutation-positive unresectable or metastatic melanoma, by measuring finger-prick DBS samples. In this case, $K_{BC/pla}$ was determined using the percentage of vemurafenib bound to blood cells described in the literature (11.4%) for C_{BC} and the remaining fraction (88.6%) for C_{pla}. Thus, $K_{BC/pla}$ was 0.129 and was used in Eq. (12) along with individual Hct. The comparison between EC_{pla} and actual plasma concentrations was evaluated in 43 paired plasma and DBS samples, obtained from 8 patients with melanoma. Deming regression and Bland-Altman plot (Table II) indicated that Eq. (12) adequately predicted the actual C_{pla}. (Table II). To avoid the need for Hct measurement for each sample, the authors also proposed to calculate EC_{pla} using the slope value obtained from the correlation between C_{DBS} and actual plasma concentration (section 5.4).

Alternatively to Eqs. (11) and (12), Antunes *et al.* (41) estimated plasma concentration using Hct and F_p, with the following simplified formula [16]:

$$EC_{pla} = \frac{C_{DBS}}{1 - Hct}*F_p \quad (16)$$

They developed a LC-MS/MS method for the quantification of imatinib in finger-prick DBS samples collected from 50 patients affected by CML. Authors applied Eq. (16) based on the data reported by Kretz *et al.* (66): they demonstrated a significant partition of imatinib into blood cells and measured an average F_p of 0.70 in healthy humans (Hct in the range of 44–47%) and an average F_p of 0.81 in acute lymphatic leukemia patients (Hct in the range of 29–37%). Antunes *et al.* recalculated the F_p using Eq. (16) and substituting EC_{pla} with the actual C_{pla} obtained from their patients' plasma samples. Thus, Eq. (16) was applied using the individual Hct and the calculated F_p value of 0.83. After Eq. (16) application, a high correlation between EC_{pla} and actual plasma concentration was obtained (Table II). In addition, EC_{pla} was also obtained using an empirically calculated CF (section 5.3).

The same simplified Eq. (16) was exploited by Hahn *et al.* (36) in their LC method with fluorescence (FL) detection for the quantification of irinotecan and its metabolite SN-38 (they used acid conditions to prevent the carboxylate form formation of both compounds) in finger-pricks DBS. The authors reported the comparison between analytesC_{DBS} and C_{pla} using 19 paired DBS and plasma samples collected from patients treated with irinotecan in single or combined chemotherapy regimens, 1 h and 24 h after the beginning of the infusion to finally calculate the drug AUC (area under the concentration-vs-time curve). The calculated F_p values were different for 1 h and 24 h post-infusion samples: 0.37 and 0.22 for irinotecan and 0.54 and 0.59 for SN-38, respectively. These values were in line with the different plasma protein binding of irinotecan (65%) and SN-38 (95%) since only the unbound fraction of the drug in plasma is able to partition into erythrocytes. C_{pla} and C_{DBS} showed a higher correlation at the collection time of 1 h compared to those obtained at 24 h post infusion for both irinotecan and SN38 (Table II). Other conversion methods (CF and regression equation application) were applied and gave better results (Table II) than those obtained with Hct- and F_p- based normalization, as reported in the following sections (5.3 and 5.4).

Table II Overview of DBS to Plasma Concentration Conversion Methods Applied in the Literature for Specific Drugs

Analyte(s)	Ref	DBS-method validation	C_{DBS} vs C_{pla}	Correlation method	Deming/Passing-Bablok regression	Bland-Altman plot	EMA/FDA acceptance criteria*
nilotinib	(43)	FDA guidelines (49); internal bioanalytical method validation guidelines (59–61)	$C_{DBS\ (FP)}$: 144–1518 μg/L C_{pla}: 376–2663 μg/L	Hct + F_p + f_u (Individual Hct)	Deming: EC_{pla}: 232–2745 μg/L m: 0.96 ± 0.05 (95% CI, 0.86 to 1.06); q: −83.12 ± 44.35 (95% CI, −172.90 to 6.66)	BIAS: −14.3% ± 2.8% (range: −56% - 24%) (95% CI, −19.9% to −8.8%); 95%	73%
				Hct + F_p + f_u (Mean Hct)	EC_{pla}: 243–2562 μg/L m: 0.95 ± 0.04 (95% CI, 0.86 to 1.03); q: −70.26 ± 43.52 (95% CI, −158.36 to 17.84)	BIAS: −14.0% ± 2.6% (95% CI, −19.3% to −8.7%).	85%
				SA	EC_{pla}: 331–2780 μg/L m: 1.00 ± 0.04 (95% CI, 0.91 to 1.09); q: 0.34 ± 46.27 (95% CI, −93.34 to 94.01)	BIAS: −0.6 ± 2.3% (range: −32.9% - 35.4%) (95% CI, −5.3% to 4.1%)	80%
	(40)[a]	FDA guideline(49), recommendations and guidelines for DBS method use in regulated bioanalysis (62,63)	$C_{DBS\ (V)}$: 586–860 μg/L C_{pla}: N/A	Hct [a]	Linear regression R^2 = 0.9772; m: 1.102; q: −10.818	BIAS: −7.8% (95% CI, −22.6% to 7.1%)	N/A
imatinib	(40)[a]	FDA guideline(49), recommendations and guidelines for DBS method use in regulated bioanalysis (62,63)	$C_{DBS\ (V)}$: 586–860 μg/L C_{pla}: N/A	Hct [a]	Linear regression R^2 = 0.9772; m: 1.102; q: −10.818	BIAS: −7.8% (95% CI, −22.6% to 7.1%)	N/A
	(41)	EMA guideline, internal bioanalytical method validation guidelines (50,59,64)	$C_{DBS\ (FP)}$: 50.3–3074 ng/mL, C_{pla}: 62.5–4169.2 ng/mL (r = 0.96, $p < 0.01$)	Hct + F_p	Passing-Bablok EC_{pla}: 66.6–3943.5 ng/mL, r = 0.96, p < 0.01 m: 95% CI of 0.91 to 1.09; q: 95% CI, −70.76 to 101.43	BIAS: 15.7 ng/mL (95% CI, −457.3 to 488.7 ng/mL)	100% (range: 82–109%)
				CF	EC_{pla}: 64.4–3934.8 ng/mL r = 0.97, p < 0.01 m: 95% CI, 0.92 to 1.05; q: 95% CI, −85.96 to 82.83	BIAS: 49.9 ng/mL (95% CI, −372.1 to 471.8 ng/mL)	100%
	(42)	FDA/EMA guidelines (49,50) EBF recommendation (6)	*(IMA)* $C_{DBS\ (V)}$: 183–3340 ng/mL; C_{pla}: 310–5840 ng/mL *(NOR-IMA)* $C_{DBS\ (V)}$: 45–409 ng/mL; C_{pla}: 67–672 ng/mL	Hct	Passing-Bablok *(IMA)* m: 0.86 (95% CI, 0.77 to 0.94); q: 77.1 (95% CI, 1.7 to 181.0) *(NOR-IMA)* m: 0.97 (95% CI, 0.89–1.06); q: 10.5 (95% CI, −5.6 to 31.4)	*(IMA)* BIAS: −101 (95%CI, −444 to 242); *(NOR-IMA)* BIAS: −2 (95% CI, −83 to 79);	*(IMA)* 89.0% *(NOR-IMA)* 78.2%
				CF	*(IMA)* m: 0.96 (95% CI, 0.91 to 1.01); q: 53.3 (95% CI, 0.0 to 107.4) *(NOR-IMA)* m: 1.00 (95% CI, 0.92 to	*(IMA)* BIAS: −4 (95% CI, −275 to 267); *(NOR-IMA)* BIAS: −1 (95% CI, −75 to 77);	*(IMA)* 92.7% *(NOR-IMA)* 83.6%

Table II (continued)

Analyte(s)	Ref	DBS-method validation	C_{DBS} vs C_{pla}	Correlation method	Deming/Passing-Bablok regression	Bland-Altman plot	EMA/FDA acceptance criteria*
					1.09); q: 1.8 (95% CI, 0.9 to 1.1)		
tamoxifen	(34)	FDA/EMA guidelines(49,50); EBF recommendations (6,65)	*(Tamoxifen)* $C_{DBS\ (FP)}$: 51.0–176 ng/mL; C_{serum}: 54.7–231 ng/mL *(EDF)* $C_{DBS\ (FP)}$: 1.61–12.2 ng/mL; C_{serum}:2.12–21.6 ng/mL	$Hct + F_p$	Deming: *(Tamoxifen)* m: 1.04 (95% CI, 0.89 to 1.19); q: 4.39 (95% CI, −11.7 to 20.5) *(EDF)* m: 0.99 (95% CI, 0.93 to 1.05); q: 0.05 (95% CI, −0.31 to 0.41)	*(Tamoxifen)* BIAS: 8.72 ng/mL *(EDF)* BIAS: 0.042 ng/mL	*(Tamoxifen)* 84.1% *(EDF)* 100%
	(35)	EMA guidelines (50), EBF recommendations and internal bioanalytical method validation guidelines (6,59)	*(Tamoxifen)* $C_{DBS\ (FP)}$: 40.0–290.0 ng/mL; C_{pla}: 52.6–307.8 ng/mL; *(NDT)* $C_{DBS\ (FP)}$: 76.3–504.0 ng/mL; C_{pla}: 85.6–575.9 ng/mL; *(EDF)* $C_{DBS\ (FP)}$: 1.0–24.2 ng/mL; C_{pla}: 2.4–40.3 ng/mL; *(HTF)* $C_{DBS\ (FP)}$: 0.5–3.2 ng/mL; C_{pla}: 0.7–3.9 ng/mL)	Hct + CF	Passing-Bablok *(Tamoxifen)* EC_{pla}: 49.9–364.3 ng/mL; m: 95% CI, 0.8343 to 1.1120; q: 95% CI, −11.8954 to 17.8583 *(NDT)* EC_{pla}: 104.4–664.3 ng/mL; m: 95% CI, 0.9113 to 1.1465; q: 95% CI, −30.1816 to 26.4940 *(EDF)* EC_{pla}: 1.5–41.3 ng/mL; m: 95% CI, 0.9205 to 1.1744; q: 95% CI, −1.0641 to 0.7439 *(HTF)* EC_{pla}: 0.5–4.4 ng/mL; m: 95% CI, 0.9289 to 1.1778; q: 95% CI, 0.1460 to 0.1349 (individual)	*(Tamoxifen)* BIAS: 4.0 ng/mL (95% CI, −42.4 to 50.4 ng/mL) *(NDT)* BIAS: −0.7 ng/mL (95% CI, −108.6 to 107.2 ng/mL) *(EDF) BIAS*: −0.0 ng/mL (95% CI, −7.1 to 7.0 ng/mL) *(HTF)* BIAS: 0.06 ng/mL (95% CI, −0.77 to 0.90 ng/mL)	97% (*Tamoxifen*); 95% (*NDT*); 80% (*EDF*); 88% (*HTF*)
pazopanib	(44)	N/A	$C_{DBS\ (FP)}$ were on average 48.0% (SD, 8.5%) lower than C_{pla}	Hct		Passing-Bablok m: 1.15 (95% CI, 1.04 to 1.24); q: −8.53 (95% CI, −12.22 to −4.41)	BIAS: −2.4 μg/mL (range: −19.2 - 13.2 μg/mL; SD 6.8 μg/mL)
				within ±25% 92.6%			
	Hct	(fixed)	m: 1.17 (95% CI, 1.07 to 1.26); q: −9.67 (95% CI, −13.28 to −5.51)	BIAS: −2.0 μg/mL (range: −18.7 - 16.7 μg/mL; SD 7.1 μg/mL)	87.4%		
(45)	FD-	A/EMAguidelines (49,50); GLP; EBF recommendations (6,65)	$C_{DBS\ (FP)}$ vs C_{pla} $R^2 = 0.872$	SA	Deming, m: 1.00; q:-0.123; Pearson's r = 0.934	BIAS: 0.08 μg/mL	79.2%
vemurafenib	(37)		r = 0.964		Deming	N/A	97%

Table II (continued)

Analyte(s)	Ref	DBS-method validation	C_{DBS} vs C_{pla}	Correlation method	Deming/Passing-Bablok regression	Bland-Altman plot	EMA/FDA acceptance criteria*
		FDA/EMA guidelines (49,50)		Hct + $K_{BC/pla}$	m: 1.03 (95% CI, 0.96 to 1.09); q: −2.75 μg/mL (95% CI, −4.61 to −0.89 μg/mL) Pearson's r = 0.939		
				SA	m: 1.00 (95% CI, 0.94 to 1.07); q: −1.42 μg/mL (95% CI, −3.31 to 0.48 μg/mL) Pearson's r = 0.963	N/A	100%
dasatinib	(40)[a]	FDA guideline (49), recommendations and guidelines for DBS method use in regulated bioanalysis (62,63)	$C_{DBS\ (V)}$: 586–860 μg/L C_{pla}: N/A	Hct [a]	Linear regression R^2 = 0.9772; m: 1.102; q: −10.818	BIAS: −7.8% (95% CI, −22.6% to 7.1%)	N/A
etoposide	(33)	FDA guidelines(49); Hct effect	R^2 = 0.9753	Hct	Deming: m: 1.023 ± 0.082; q: −0.3530 μg/mL ±0.481 μg/mL; R^2 = 0.9726	BIAS: −0.241 μg/mL (95% CI, −0.618 to 0.136); 95% LoA: −1.88 to 1.40	N/A
paclitaxel	(38)	FDA guidelines (49); impact of Hct; impact of spotted blood volume	*(A*)* $C_{DBS\ (FP)}$: 11.7–83.1 ng/mL; C_{pla}: 10.9–49.6 ng/mL; r = 0.930; $C_{pla}/C_{DBS\ (FP)}$ = 0.707 *(B*)* $C_{DBS\ (FP)}$: 51.6–220.9 ng/mL; C_{pla}: 60.9–249.2 ng/mL; r = 0.896; $C_{pla}/C_{DBS\ (FP)}$ = 0.904	Hct + F_p	N/A	N/A	within ±25%: *(A*)* 78.9% *(B*)* 86.7%
				CF	Passing-Bablok, m: 95% CI, 0.9805–1.1717; q: −4.6371 - 2.8749, (*P* = 0.42)	BIAS: 1.4, 95% CI, −30.6% to 33.4%)	*(A*)* 94.7% *(B*)* 86.7%
				SA	N/A	N/A	*(A*)* 100% *(B*)* 80.0%
Irinotecan	(36)	FDA guidelines (49); impact of Hct	*(Irinotecan)* $C_{DBS\ (FP)}$: 1091–5213 ng/mL (1 h), 38.4–393.1 ng/mL (24 h); C_{pla}: 606–2802 ng/mL (1 h), 12.8–110.0 ng/mL (24 h) r = 0.949 (1 h); 0.766 (24 h) *(SN-38)* $C_{DBS\ (FP)}$: 3.45–18.93 ng/mL (1 h), 0.56–3.08 ng/mL (24 h); C_{pla}: 3.09–19.29 ng/mL (1 h), 0.58–2.97 ng/mL (24 h) r = 0.933 (1 h) 0.796 (24 h)	Hct + F_p	N/A	N/A	*(Irinotecan)* 73.7% (1 h); 32% (24 h) *(SN-38)* 68.4% (1 h); 63.2% (24 h)
				CF	N/A	N/A	*(Irinotecan)* 78.9% (1 h); 37% (24 h) *(SN-38)* 84.2% (1 h); 68.4% (24 h)
				SA	N/A	N/A	*(Irinotecan)* 84.2% (1 h); 53% (24 h)

Table II (continued)

Analyte(s)	Ref	DBS-method validation	C_{DBS} vs C_{pla}	Correlation method	Deming/Passing-Bablok regression	Bland-Altman plot	EMA/FDA acceptance criteria*
							(SN-38) 78.9% (1 h); 68.4% (24 h)
docetaxel	(39)	FDA guidelines (49); impact of Hct	*(5 ± 5 min)* $C_{DBS\ (FP)}$: 756–3047 ng/mL; $C_{DBS\ (V)}$: 676–2940 ng/mL; C_{pla}: 779–3842 ng/mL *(60 ± 10 min)* $C_{DBS\ (FP)}$: 57–331 ng/mL; $C_{DBS\ (V)}$: 59–316 ng/mL; C_{pla}: 50–395 ng/mL $C_{DBS\ (FP)}$ vs C_{pla}: r = 0.92; AUC: 2.4–4.9 mg h/L	Hct + F_p	Passing-Bablok *(5 ± 5 min)* EC_{pla}: 794–3606 ng/mL *(60 ± 10 min)* EC_{pla}: 50–208 ng/mL m: 95% CI, 0.909 to 1.007; q: 95% CI, −12.625 to 15.124 AUC: 2.3–4.8 mg h/L; m: 95% CI, 0.75 to 1.20; q: 95% CI, −0.600 to 0.725	AUC BIAS: 0.2%, 95% CI, −17% - 17.5%	within ±15% AUC: 93.5%
				CF	*(5 ± 5 min)* EC_{pla}: 757–3407 ng/mL *(60 ± 10 min)* EC_{pla}: 49–225 ng/mL m: 95% CI, 0.935–1.092; q: 95% CI, −15.741 - 18.388 AUC: 2.4–4.2 mg h/L; m: 95% CI, 0.68 to 1.14; q: 95% CI, −0.414 - 0.928	AUC BIAS: 0.1%, 95% CI, −18.1% - 18.3%	AUC: 90%
radotinib	(47)	FDA/EMA guidelines (49,50)	$C_{DBS\ (FP)}$: 179–2290 ng/mL; C_{pla}: 260–3190 ng/mL; $r^2 = 0.97$ C_{DBS}/C_{pla}: 0.75	Hct	Deming regression EC_{pla}: 234–3013 ng/mL; $r^2 = 0.97$; m: 1.02; q: −1.80 ng/mL	BIAS: −1.60% ± 11.3% (SD) (95% LoA, −23.7% to 20.5%)	93.3%
				SA	C_{pla}: 240–3069 ng/mL; $r^2 = 0.97$; m: 0.99; q: 4.26 ng/mL	BIAS: −0.44% ± 11.2% (SD) (95% LoA, −22.4% to 21.5%)	93.3%

[a] value obtained for imatinib, dasatinib and nilotinib samples together;

*The difference in concentration between the two methods should be within 20% of the mean difference for at least 67% of the samples;

*A** patients treated with weekly paclitaxel, *AUC* Area Under the Curve, *B** patients treated with three weekly paclitaxel, *CDBS* dried blood spot concentration, *CDBS (FP)* dried blood spot from finger prick concentration, *CDBS (V)* venous dried blood spot concentration, *CF* correction factor, *CI* confidence interval, *Cpla* plasma concentration, *Cserum* serum concentration, *DBS* Dried Blood Spot, *DC* direct correlation, *EBF* European Bioanalysis Forum, *ECPLA* estimated plasma concentration, *EDF* (*Z*)-Endoxifen, *EMA* European Medicine Agency, *FDA* Food and Drug Administration, *Fp* blood-to-plasma drug concentration ratio, *fu*-unbound drug fraction in plasma, *GLP* Good Laboratory Practice, *Hct* hematocrit, *HTF* 4-hydroxytamoxifen, *IMA* imatinib, *KBC/pla* blood-cell to plasma partition coefficient, *LoA* Limit of Agreement, *m* slope, *N/A* not applicable, *NDT* N-desmethyltamoxifen, *NOR-IMA* nor-imatinib, *q* intercept, *SA* statistical analysis, *SD* Standard Deviation

The same three approaches (method 1, which is discussed in this section, method 2 in section 5.3 and method 3 in section 5.4) applied by Hahn *et al.* (36) were used by Andriguetti *et al.* (38) in their LC-MS/MS quantification method for paclitaxel in DBS. In this study, paclitaxel quantification was performed in plasma, DBS from finger-prick and venous DBS obtained from patients treated with weekly (19, group A) and three weekly (15, group B) paclitaxel. The study population was divided into two groups according to the formulation administered as Cremophor EL® (CrEL, a pharmaceutical vehicle used to dissolve paclitaxel for intravenous administration) affected drug disposition by entrapping the compound in micelles, thus reducing its free fraction and affecting the $K_{BC/pla}$ (67). Moreover, CrEL clearance is highly influenced

by infusion duration: prolonging the infusion from 1 h (group A) to 3 h (group B), CrEL clearance approximately doubles (68). This phenomenon leads to a decrease in CrEL systemic levels and a consequent lower influence on the calculated paclitaxel EC_{pla}. Since C_{DBS} from finger-prick samples was highly correlated to that obtained from venous blood DBS, estimation of plasma concentration was conducted on finger-prick DBS. Authors evidenced a higher correlation between C_{pla} and C_{DBS} collected from group B ($r = 0.930$) rather than those from group A ($r = 0.896$). With method 1, EC_{pla} was calculated with Eq. (16), where F_p was calculated as previously reported (36, 41). The calculated F_p was different between the two patients' groups: 0.5 for group A and 0.56 for group B. As a result, EC_{pla} was within ±25% of the measured plasma concentration in 79% of the samples collected from group A and in 87% of the samples from group B. The other two approaches had better performance to predict EC_{pla} from C_{DBS}, as reported in following sections.

Raymundo *et al.* (39), described a LC-MS/MS method for the quantification of docetaxel in DBS from both finger-prick and venous blood. This method was applied for the quantification of this anticancer drug in 31 patients affected by different solid tumors and treated with docetaxel as monotherapy or in combination with other drugs. Two samples were collected from each patient: (5 ± 5 min before and 60 ± 10 min after the end of the infusion) to calculate docetaxel AUC (used as target for docetaxel TDM). As for paclitaxel, docetaxel formulation includes Tween 80 as an excipient to improve its solubility. This compound modifies the distribution of docetaxel in blood (69), therefore this effect should be considered to correlate DBS and plasma concentrations. In fact, experiments both *in vitro* and *in vivo* (69) showed a direct proportionality between the concentration of Tween 80 and docetaxel F_p. EC_{pla} was estimated using two strategies reported in previous studies (36, 38, 41): 1) application of Eq. (16) (where F_p was calculated using this equation and the actual plasma concentrations) and 2) application of an experimentally obtained CF (reported in section 5.2). Authors reported that finger-prick C_{DBS} from the first sampling was on average 89% of the plasma concentration while C_{DBS} from the second sampling was 140% of the plasma level, suggesting a concentration- or time-dependent blood cells uptake. Using the Hct- and F_p-based normalization to calculate EC_{pla} (F_p was 0.71 and 0.44 for the first and second time point samples, respectively), no systemic or proportional errors were revealed by Passing-Bablok (Table II) and 93.5% of AUC calculated from EC_{pla} were within ±15% of those obtained from plasma samples.

Hct-Based Normalization

As reported in section 4, when a drug is not partitioned in blood cells and is almost only present in the plasma compartment, plasma concentration can be estimated based on the C_{DBS} measured and the Hct value, using Eq. (10). Several methods (33, 40, 42, 44, 47) reported in Table I applied this equation since the authors observed no plasma-to-blood partitioning for the analyzed drugs (etoposide, imatinib, norimatinib, radotinib, nilotinib, dasatinib, pazopanib) and most of these compounds were reported to be in plasma (i.e., for imatinib the blood-to-plasma partition ratio was 0.45 and for radotinib it was 0.75). In the case of pazopanib (44) the use of Hct-based normalization was also justified by observing that only unbound fraction of a drug can partition in blood cells and, since pazopanib has a high protein binding (>99.9%), the unbound fraction could be considered negligible.

A first method was developed by Kralj *et al.* (40) for the quantification of three TKIs: imatinib, dasatinib and nilotinib. The Hct-based normalization using Eq. (10) was applied on a set of 23 venous DBS samples: 18 for imatinib, 3 for dasatinib and 2 for nilotinib. Considering the three TKIs together, the obtained EC_{pla} showed a high correlation ($R^2 = 0.9772$) with the actual plasma concentrations (Table II). Despite the authors analyzed only venous DBS, they considered the method applicable in clinic, and thus in finger-prick DBS samples, due to the fact that differences between capillary and venous blood can arise during absorption phase of low molecular size, high lipid solubility and relatively low protein binging drugs: characteristics not related to TKIs.

Likewise, in the LC-MS/MS method proposed by De Wit *et al.* (44) for pazopanib quantification in DBS, the correlation between DBS and plasma concentrations was conducted on a small set of samples composed by 12 patients. Concentrations measured in finger-prick and venous blood DBS were found in good agreement with each other (Table II). Interestingly, EC_{pla} was calculated using both individual Hct values and fixed Hct values (0.40 L/L and 0.45 L/L for men and women, respectively) and no significant differences between both approaches were observed (Table II). Authors used as acceptance criteria the 25% range around the found ratio of the two methods (DBS and plasma) and 92.6% (87.4% with fixed Hct) of EC_{pla} were within this clinical acceptance limit. It has been considered clinically relevant since pazopanib can be dose-adjusted in steps of 25% of the total dose.

The LC method with FL detection proposed by Rezonja *et al.* (33) for etoposide quantification in venous DBS was applied to a slightly larger set of samples (28) collected from patients affected by small cell lung cancer (SCLC) in treatment with etoposide and platinum. The EC_{pla} was obtained applying Eq. (10) and individual Hct: obtained EC_{pla} were comparable to measured plasma concentrations, as shown by Deming regression and Bland Altman test (Table II).

Fifty-five samples collected from 26 patients (affected by gastrointestinal stromal tumor (GIST) and treated with imatinib) were used to evaluate the agreement between imatinib

and norimatinib (active metabolite) EC_{pla} and corresponding plasma concentrations in the analysis conducted by Iacuzzi *et al.* (42). EC_{pla} was calculated from C_{DBS} by means of 2 strategies: 1) applying Eq. (10) with individual Hct and 2) using a CF (section 5.3). A high correlation between finger-prick and venous DBS was found to demonstrate that the two samples were equivalent ($R^2 = 0.9967$ for imatinib and $R^2 = 0.9798$ for norimatinib). Applying Eq. (10), a good agreement between the two methods (plasma and DBS normalized by Hct) was obtained according to EMA/FDA guidelines: 89% (49/55) and 78% (43/55) of EC_{pla} resulted within ± 20% of the mean for imatinib and norimatinib, respectively. Both Passing-Bablok regression and Bland-Altman analyses confirmed these results, as reported in Table II.

A different approach was proposed by Lee *et al.* (48) in their LC-MS/MS method for the quantification of radotinib in finger-prick DBS. A second-degree polynomial function (Eq. 17) was applied to obtain EC_{pla} from 45 samples collected from patients affected by CML:

$$EC_{pla} = \frac{C_{DBS}}{\left(1 - Hct + Hct^2\right)} \tag{17}$$

This function was obtained by empirically inserting in the following equation

$$EC_{pla} = \frac{C_{DBS}}{\left(a + b*Hct + c*Hct^2\right)} \tag{18}$$

the Hct measured from each patient and different combinations of values (from −1 to 1) for each term a, b and c. Among the 125 sets of polynomial combinations tested, Eq. (17) resulted the best outcome. In fact, whit this function, they reached a high correlation between C_{pla} and EC_{pla}, with a $R^2 = 0.97$ (Table II). The difference between EC_{pla} and C_{pla} was within ±20% in 93.3% of samples, meeting the FDA/EMA acceptance criteria. Finally, EC_{pla} was also directly calculated using the Deming regression between C_{DBS} and actual plasma concentrations (section 5.4).

Normalization for a Correction Factor Experimentally Determined

Another way of converting DBS measurement to EC_{pla} is to calculate the mean ratio between actual plasma concentrations and DBS concentrations from a population and to use this ratio as a correction factor. This approach requires data from clinical validations of many patients and awareness that extreme individual Hct can create bias. CF is experimentally determined by the following Eq. (19):

$$CF = \frac{C_{pla}}{C_{DBS}} \tag{19}$$

Then, the estimated plasma value is calculated from the concentration obtained with the DBS analysis according to Eq. (20):

$$EC_{pla} = C_{DBS}*CF \tag{20}$$

Equation (20) was applied in the LC-MS/MS method for the quantification of imatinib in DBS samples collected from patients (50) affected by CML and already discussed in section 5.1 (41). EC_{pla} was firstly obtained using Hct- and F_p- based normalization. The difference between the calculated EC_{pla} and measured plasma concentration was within ±20% of their mean for all samples. To avoid the use of individual Hct, EC_{pla} was also estimated using a CF calculated with Eq. (19), obtaining a value of 1.28. Using this approach, a high correlation between EC_{pla} and actual plasma concentration was achieved, and the two values were comparable (Table II).

The same group (35) reported a LC-MS/MS method for the quantification of tamoxifen and its metabolites N-desmethyl tamoxifen (NDT), 4-hydroxytamoxifen (HTF) and endoxifen (EDF) in finger-prick DBS samples. In this study, the correlation between the drug or metabolites' C_{DBS} and actual plasma concentration was conducted on 91 paired DBS and plasma samples from patients treated with tamoxifen and affected by breast cancer. They applied the following Eq. (21):

$$EC_{pla} = \frac{C_{DBS}}{1 - Hct}*CF2 \tag{21}$$

where the individual Hct was used and CF2 was obtained by Eq. (22):

$$CF2 = \frac{C_{DBS}}{C_{pla}} \tag{22}$$

The calculated CF2s were 0.84, 0.78, 1.12, 0.87 for tamoxifen, NDT, EDF and HTF, respectively. The authors obtained a good correlation between EC_{pla} and the measured plasma concentration (Table II): the difference between the two values was within ±20% in 97% (tamoxifen), 95% (NDT), 80% (EDF) and 88% (HTF) of the analyzed samples, being in accordance with EMA acceptance criteria.

The LC-MS/MS method proposed by Raymundo *et al.* (39) was applied for docetaxel quantification in DBS from 31 patients affected by prostate, breast or lung cancer. As already reported in section 5.1, two samples were collected for each patient: 5 ± 5 min before and 60 ± 10 min after the end of the infusion. The first approach applied the Hct- and F_p-based normalization (Eq. 16, while the second approach adjusted the C_{DBS} with a CF2, (Eq. 22), introduced by Antunes *et al.* in the previous study (35). The CF2 for docetaxel were 1.12 and 0.68 for the first and second set of patient samples, respectively. Despite Passing-Bablok regression indicated the absence of constant error and 90% of the AUC

measurements calculated from EC_{pla} were within ±15% of those obtained from plasma samples, the authors clarified that the effectiveness of these CF2s application needs to be further demonstrated.

The CF approach for the estimation of EC_{pla} was applied also by Hahn *et al.* (36) in the method for the quantification of irinotecan and SN38 in DBS from finger-prick. The analysis was conducted on 19 paired DBS and plasma samples collected 1 h and 24 h after the beginning of the infusion from patients treated with irinotecan. In this case, EC_{pla} was obtained using the application of a CF (Eqs. (19) and (20)). The calculated CF values were different for samples collected 1 or 24 h after the beginning of the infusion: for irinotecan CFs were 0.57 (range 0.44–0.85) and 0.39 (range 0.17–0.72), respectively, while for SN38 CFs were 0.85 (range 0.57–1.08) and 0.97 (range 0.60–1.64), respectively. The concordance between EC_{pla} and actual plasma measurements was slightly increased with method 2 respect to method 1 (Table II), but still poor. Better results were obtained with method 3 (with the application of regression analysis, section 5.4).

Similarly, three methods were applied to calculate EC_{pla} in the LC-MS/MS quantification method for paclitaxel in DBS proposed by Andriguetti *et al.* (38). The drug quantification was conducted on DBS (finger-prick and venous) collected from 19 patients treated with weekly paclitaxel scheme (group A) and 15 patients treated with three weekly paclitaxel scheme (group B). Using Hct- and Fp- based normalization, EC_{pla} was within ±25% of the measured plasma concentration in 79% (group A) and in 87% (group B) of the analyzed samples. By applying two CFs (0.707 for group A and 0.904 for group B), calculated with Eq. (19), the calculated EC_{pla} was within 25% of the actual plasma concentration in 95% (group A) and 87% of measured samples (Table II). Due to its better predictive performance, only for this last approach authors analyzed the correlation between the C_{pla} and EC_{pla} with Passing-Bablok regression and Bland-Altman plot and no systematic or proportional differences between the two concentrations were observed (Table II).

Equation (19) was applied to calculate the CFs to estimate the EC_{pla} in the LC-MS/MS method for the quantification of imatinib and its active metabolite in DBS proposed by Iacuzzi *et al.* (42). EC_{pla} was also calculated using Eq. (10) obtaining a good correlation with plasma values. Better results were obtained with the application of the calculated CF (1.73 for imatinib and 1.61 for norimatinib) (Table II), compared to the results obtained using Eq. (10) (section 5.2). The slope of Passing-Bablok regression was nearly equal to 1 for both analytes, while no constant errors resulted for both the analytes from the intercept values (Table II). Among all methods reported in this review, only a further validation of this strategy using an independent set of samples (12) is herein reported. The agreement between concentrations in plasma and in DBS samples, after the CF normalization, was verified: 100% of EC_{pla} resulted within ±20% of the mean for both imatinib and norimatinib with a good linearity with plasma concentrations (R^2 = 0.9895 and 0.9474 for imatinib and norimatinib, respectively).

Normalization through Statistical Analysis

Besides the application of the formulas analyzed so far (section 5.1, 5.2 and 5.3), in some cases, normalization was carried out through the application of statistical analyses, in particular, through the use of linear regression analyses. As reported for the application of a CF, this method is characterized by the advantage to estimate plasma concentrations from DBS analysis without knowing patient's Hct or drug specific parameters (such as F_p or $K_{BC/pla}$). On the other hand, particular attention should be addressed to patients' characteristics (such as Hct range) when the method needs to be translated to a different or a more extended population.

The first case considered was the method proposed by Lee *et al.* (47), already discussed in session 5.1 (Hct-based normalization). EC_{pla} of 45 samples from patients with CML in treatment with radotinib were also directly calculated using the Deming regression between C_{DBS} and actual plasma concentrations ($EC_{pla} = 1.34C_{DBS} + 0.78$). As a result, in more than 93% of the EC_{pla} and C_{pla} pairs the difference of the two values fell within ±20% of the mean (Table II). Nonetheless, the authors suggested to apply the "direct" prediction only to patients with similar characteristics to those of their study to avoid unpredictable confounding factors.

Another LC-MS/MS method that used statistical analysis to predict EC_{pla} is that of Boons *et al.* (43) for the quantification of nilotinib in DBS samples collected from 20 patients affected by CML. As already reported in section 5.1, EC_{pla} was predicted from C_{DBS} using three methods: application of Eq. (16) with both (1) individual and (2) mean Hct value and (3) application of the bias between plasma and DBS concentrations. In this latter case, EC_{pla} was predicted using the constant (b = −41.68) and proportional (m = 0.56) bias from the Deming fit between the actual plasma concentration and C_{DBS} (Eq. 23):

$$EC_{pla} = \frac{C_{DBS} + b}{m} \tag{23}$$

The agreement between EC_{pla} and actual plasma concentration was high, as shown by Deming regression and Bland Altman test (Table II). This approach appeared to be the most accurate to predict plasma concentrations.

The same Eq. (23) obtained from Deming regression analysis and applied in the previous study was also used in a LC-MS/MS method for the quantification of pazopanib in 221 finger-prick DBS samples collected from patients affected by advanced solid tumors and treated with this drug (45). Based on the weighted Deming fit (R^2 = 0.872) of C_{DBS} and actual

plasma concentration, EC_{pla} was calculated using the following data: b = 0.182 and m = 0.709. EC_{pla} were within ±20% of measured plasma concentrations for 79.2% of DBS samples. Moreover, when used to identify patients above or below the pazopanib threshold (20 µg/mL) for TDM purposes, the plasma and DBS methods were in agreement in 91.4% of the cases. Correction for individual Hct did not improve the correlation between EC_{pla} and measured plasma concentrations compared to the empirical Deming regression formula. Authors suggested that Hct, even though influences the analytical results (as reported in the DBS-specific validation study), is not the most important factor driving the variability between the two methods in the clinical setting.

Nijenhuis *et al.* (37) proposed to obtain vemurafenib EC_{pla} using both Eq. (12), (section 5.1, method 1), and the slope value obtained from the correlation between C_{DBS} and actual plasma concentration (Eq. 24, method 2):

$$EC_{pla} = \frac{C_{DBS}}{m} \tag{24}$$

The analysis was performed on 43 paired plasma and DBS samples obtained from 8 patients with melanoma.

As already reported, a strong correlation was found between C_{DBS} and measured C_{pla} (Table II). Method 2 adequately predicted the actual C_{pla} as already demonstrated for method 1 with the advantage to avoid the use of individual Hct (Table II). Differences between EC_{pla} and the analyzed plasma concentrations were within ±20% for all samples.

Three different approaches (method 1, 2 and 3) for EC_{pla} estimation were applied by Hahn *et al.* (36) in a LC-FL method for the quantification of irinotecan and its active metabolite SN-38 in finger-pick DBS. Method 1 based on individual Hct and F_p (section 5.1), method 2 consisted in the calculation of a CF (section 5.3) and method 3 based on the application of the regression equation between C_{pla} and C_{DBS} (for irinotecan: y = 0.463x + 253.96 and y = 0.1966x + 20.28 for measurements collected 1 h and 24 h post-infusion respectively; while for SN-38 y = 0.9947x − 1.7641 (1 h) and y = 0.6623x + 0.4524 (24 h)). The application of method 3 increased the correlation between EC_{pla} and measured plasma concentration for both irinotecan and SN38 for samples collected after 1 h, while the correlation was poorer for samples collected after 24 h (Table II). Anyway, irinotecan AUCs calculated using both plasma and EC_{pla} from method 3 were highly concordant, with r = 0.918 and 17 (over 19) patients had concordant classification when using plasma or DBS levels to calculate AUC. Authors concluded that additional studies, with larger groups of patients, are necessary to evaluate the feasibility of the quantification of irinotecan and SN38 in DBS matrix for dose individualization purpose.

Andriguetti *et al.*(38), reported a LC-MS/MS quantification method for paclitaxel in DBS. This method was applied for the quantification of DBS (finger-prick and venous) collected from 19 patients treated with weekly paclitaxel scheme (group A) and 15 patients treated with three weekly paclitaxel scheme (group B). EC_{pla} was estimated with method 1 (Hct- and F_p- based normalization, section 5.1), method 2 (application of a CF, section 5.3) and method 3 (application of the regression equation). Concerning the application of method 3, the regression equations correlating C_{pla} (x-axis) and C_{DBS} (y-axis) were: $C_{DBS} = (1.725*C_{pla})-6.379$ for group A and $C_{DBS} = (1.024*C_{pla}) + 7.575$ for group B. The calculated EC_{pla} was always (100%) within 25% of actual plasma concentration for samples collected from group A while the percentage was reduced to 80% for samples collected from group B. Globally, the best predictive performance was obtained with method 2 (Table II).

Direct Correlation: When C_{DBS} Corresponds to C_{pla}

Plasma measurements can be directly replaced by DBS analysis without applying any DBS-to-plasma conversion methods when drug is equally partitioned into blood cells and plasma ($K_{BC/pla}$ and F_p are both equal to 1, Eqs. (8) and (9)). In this case, DBS and plasma concentrations are equivalent.

Irie *et al.* (46), proposed a LC-MS/MS method for the quantification of gefitinib in finger-prick DBS. Although this method was tested on only 10 dB samples collected from patients affected by non-SCLC and daily treated with gefitinib, authors reported a plasma to blood ratio of 1:1.063, with a high correlation between C_{DBS} and C_{pla} (Table III). Data obtained from Passing-Bablok and Bland-Altman (Table III) were considered proper enough to use the C_{DBS} instead of C_{pla} to evaluate the relationship with clinical outcome or toxicity. Anyway, since this study only included patients with Hct equal or lower than the normal range (not specified), authors reported that further studies in patients with a wider Hct range are needed to better evaluate the feasibility of gefitinib quantification in DBS.

Another example of direct correlation is represented by the LC-MS/MS method proposed by Matsumoto *et al.* (31) for the quantification of busulfan. This method was applied to analyze venous DBS samples collected from 10 patients at 2, 4 and 6 h after the start of the infusion. The correlations between DBS and plasma methods were conducted in terms of both busulfan concentrations and $AUC_{0-\infty}$ (area under the curve from time 0 to infinite). Differences between C_{DBS} and C_{pla}, as well as$AUC_{0-\infty}$ obtained from C_{DBS} and C_{pla} were always within ±20% (Table III).

More recently, a second study on a LC-MS/MS method for the quantification of busulfan in DBS was proposed by Dilo *et al.* (32). This method was applied for the quantification of 153 venous DBS samples collected from 15 pediatric patients who were on a 4-times daily busulfan dosing regimen before allogeneic hematopoietic stem cell transplantation

(HSCT). In this study, busulfan DBS measurements were correlated with dried plasma spot (DPS) analysis. A strong correlation between DBS and DPS busulfan measurements was observed (Table III). Interestingly, this study reported also the blood-to-plasma ratio calculation for busulfan, using a previously described method (70). Combining Eqs. (7) and (5), F_p can be expressed as follows:

$$F_p = \left(K_{BC/pla} * Hct\right) + (1 - Hct) \tag{25}$$

Where $K_{BC/pla}$ can be obtained using the following equation:

$$K_{BC/pla} = \frac{\frac{I_{BC}}{I_{BC\_ref}}}{\frac{I_{pla}}{I_{pla\_ref}}} \tag{26}$$

Where I_{BC} is the LC-MS/MS response (peak-area ratio to IS) in blood cells; I_{BC_ref} is the response for reference blood cells; I_{pla} is the response in plasma and I_{pla_ref} is the response for reference plasma. The mean ± SD of busulfan F_p obtained was 0.93 ± 0.05, in accordance with the initial hypothesis of equal distribution of the drug in blood cells and plasma.

The Case of Everolimus

Another case of direct correlation can occur when the drug preferably accumulates in blood cells compartment: this is the case of everolimus. Everolimus, an inhibitor of mammalian target of rapamycin (mTOR), is used as immunosuppressant for transplantation and in the treatment of renal cancer. It is rapidly absorbed after oral administration with a very modest oral bioavailability (5–11%) and accumulates in erythrocytes with a fixed erythrocyte to plasma accumulation ratio of 85:15 in the clinically relevant concentration range (71). The direct measurement of everolimus concentrations in plasma is highly challenging: even the minimal blood hemolysis has a large effect on the analysis, and it is not stable in plasma matrix (72). For these reasons, for clinical pharmacological studies, everolimus concentrations are routinely measured in whole blood. The whole blood-to-DBS correlation should be direct since DBS measurements represent drug concentration in whole blood. This theoretical consideration was confirmed also by experimental data.

In a first study conducted in 2009 by Van Der Heijden *et al.* (27), 5 pairs of venous blood and DBS samples from a single patient in treatment with everolimus were analyzed with a LC-MS/MS method and a higher concentration in DBS was observed than in whole blood samples, even thought the differences were not significant.

The same result was obtained in two subsequent papers of Willemsen *et al.* (28) and Veenhof *et al.* (30), where DBS and whole blood concentrations of everolimus were demonstrated to be comparable using a larger samples size (20 and 44 paired DBS and whole blood samples, respectively) (Table III).

In a single case, described by Verheijen *et al.* (29), a normalization to correlate volumetric absorptive microsampling (VAMS) and C_{WB} was applied. In this study, the analysis of 25 clinical samples collected from 10 patients was performed in both EDTA whole blood samples and VAMS. Everolimus concentrations in clinical VAMS samples were significantly higher than the C_{WB}. This outcome was consistent with previous studies conducted on caffeine, paraxanthine and paracetamol, where VAMS systematically overestimated the whole blood concentration. Weighted Deming regression was used to compare VAMS and whole blood measurements and the correlation was described quantitatively by the formula: $y = 0.691x + 0.158$, where x is the VAMS concentration and y is the C_{WB} (Table III).

AUC: Area Under the Curve; C_{DBS}: dried blood spot concentration; $C_{DBS\ (FP)}$: dried blood spot from finger prick concentration; $C_{DBS\ (V)}$: venous dried blood spot concentration; C_{DPS}: dried plasma spot concentration; CI: confidence interval; C_{pla}: plasma concentration; C_{WB}: whole blood concentration; DBS: Dried Blood Spot; EBF: European Bioanalysis Forum; EC_{pla}: Estimated plasma Concentration; EMA: European Medicine Agency; FDA; Food and Drug Administration; Hct: hematocrit; LoA: Limit of Agreement; m: slope; N/A: not applicable; PMDA: Pharmaceuticals and Medical Devices Agency (Japan); q: intercept; SA: statistical analysis; SD: Standard Deviation.

CONCLUSIONS

DBS-based methods represent an attractive option to implement TDM of anticancer drugs in clinical routine, increasing patients' compliance and simplifying pre-analytical steps. Nevertheless, their application is still limited by some aspects, such as the extensive analytical validation compared to traditional bioanalytical assays and the necessity, for most drugs, to correlate the DBS measurement to the reference value, usually established in plasma matrix. Thus, DBS-to-plasma conversion methods represent a fundamental part of the applicability evaluation of DBS sampling. In this review, the DBS-to-plasma conversion methods applied to quantitative analyses of anticancer drugs reported in the literature so far were introduced together with the relative DBS/plasma concentrations correlation studies. From the available data, some considerations can be drawn.

Firstly, the application of DBS technique for TDM is more common for drugs belonging to the kinase inhibitors (KIs) family compared to other anticancer compounds (e.g., taxanes). This could be explained since the pharmacokinetic (PK) exposure to these drugs is highly variable and, in some

Table III Overview of DBS to Plasma/Whole Blood Concentration Direct Conversion Method Applied in the Literature for Specific Drugs

Analyte(s)	Ref	DBS-method validation	C_{DBS} vs C_{pla}	Deming/Passing-Bablok regression	Bland-Altman plot	EMA/FDA acceptance criteria*
gefitinib	(46)	FDA, EMA, PMDA guidelines (49,50,51)	$C_{DBS\ (FP)}$: 52.2–386.7 ng/mL; C_{pla}: 59.7–390.5 ng/mL; $R^2 = 0.99$; $C_{pla}/C_{DBS\ (FP)} = 0.941$	Passing-Bablok: m: 0.932 (95% CI, 0.860 to 1.048);	BIAS: 6.33% (95% CI, 1.69 to 10.96)	100%
busulfan	(31)	PMDA guidelines (51)	$C_{DBS\ (V)}$: 210–1090 ng/mL; C_{pla}: 210–1097 ng/mL; $AUC_{DBS\ (V)}$: 757–1333 μ mol min/L; AUC_{pla}: 683–1320 μ mol min/L;	Linear regression: m: 0.9903; $R^2 = 0.9837$ AUC: m: 0.9986; $R^2 = 0.8752$	N/A	100% (range: 85.8%–113.2%) AUC: 100% (91.9%–110.3%)
	(32)	EMA guidelines (50)	C_{DPS}: 123.5–1695 ng/mL; C_{DBS}: 112.5–1970 ng/mL	Deming: m: 1.00 (95% CI, 0.94 to 1.00); q: −48.4 ng/mL (95% CI; −96.7 to 0.17); Pearson's r = 0.96 (95% CI, 0.95 to 0.97)	BIAS: 5.4% ± 9.6% (95% CI, 4.6 - -15.3)	100%
everolimus	(27)	FDA guidelines (49)	N/A	N/A	N/A	N/A
	(28)	EMA guidelines (50)	$C_{DBS\ (FP)}$: 3.7–33.3 μg/L; $C_{DBS\ (v)}$: 3.3–31.2 μg/L; C_{WB}: 3.6–28.5 μg/L	Passing-Bablok $C_{DBS\ (FP)}$ vs $C_{WB:}$ m: 0.89 (95% CI, 0.76 to 0.99); q: 0.02 μg/L (95% CI, −0.93 to 1.35); $r^2 = 0.95$ $C_{DBS\ (v)}$ vs $C_{WB:}$ m: 0.93 (95% CI, 0.87 to 1.04); q: −0.17 μg/L (95% CI, −1.37 to 0.51); $r^2 = 0.98$	$C_{DBS\ (FP)}$ vs $C_{WB:}$ BIAS: 0.90; 95% LoA: 0.71 to 1.08; $C_{DBS\ (v)}$ vs $C_{WB:}$ BIAS: 0.92; 95% LoA: 0.79 to 1.05	95%
	(29)	FDA/EMA guidelines (49,50); influence of Hct	N/A	Deming: m: 0.691; q: 0.158 ng/mL	BIAS: 0.6%	N/A
	(30)	FDA/EMA guidelines (49,50)	$C_{DBS\ (FP)}$: 1.9–10.9 μg/L (mean: 5.0 μg/L, SD: 2.4 μg/L); $C_{DBS\ (v)}$: 1.2–14.3 μg/L (mean: 5.4 μg/L; SD: 2.6 μg/L)	Passing-Bablok: m: 0.96 (95% CI, 0.84 to 1.06); q: 0.37 (95% CI, −0.11 to 1.99); r = 0.97	BIAS: 1.04 (95% CI, 1.00 to 1.08); 95% LoA: 0.78 to 1.30	88.6%

*The difference in concentration between the two methods should be within 20% of the mean difference for at least 67% of the samples;

**everolimus concentrations are routinely measured in whole blood, instead in plasma;

AUC Area Under the Curve, *CDBS* dried blood spot concentration, *CDBS (FP)* dried blood spot from finger prick concentration, *CDBS (V)* venous dried blood spot concentration, *CDPS* dried plasma spot concentration, *CI* confidence interval, *Cpla* plasma concentration, *CWB* whole blood concentration, *DBS* Dried Blood Spot, *EBF* European Bioanalysis Forum, *ECpla* Estimated plasma Concentration, *EMA* European Medicine Agency, *FDA* Food and Drug Administration, *Hct* hematocrit, *LoA* Limit of Agreement, *m* slope, *N/A* not applicable, *PMDA* Pharmaceuticals and Medical Devices Agency (Japan), *q* intercept, *SA* statistical analysis, *SD* Standard Deviation.

cases, a relationship between plasma concentrations and toxic/therapeutic effects (73) has already been identified. All KIs are orally administered and their bioavailability depends on gastrointestinal absorption and first-pass metabolism. Thus, these drugs are substrates of a range of drug transporters and metabolizing enzymes, the activity of which may be influenced by a series of factors, such as drug-drug interactions, genetics and food intake. Finally, some KIs are inhibitors of their own transporters and metabolizing enzymes, a property that may affect their concentration at the steady-state, possibly making it complex and unpredictable (74). For these reasons, TDM of KIs drugs as routine clinical practice is highly encouraged.

Although in a considerable number of published DBS-based methods (papers $n = 18$) no DBS/plasma (or whole blood) concentrations correlation study was reported, this tendency is most evident in older papers. Relating to methods performing a complete clinical validation (papers $n = 21$), four principal DBS-to-plasma concentrations conversion approaches can be outlined. The most general normalization (papers $n = 8$) considers both patient's Hct and blood-to-plasma partitioning (expressed as $K_{BC/pla}$ or F_p) and was

commonly applied for drugs variably distributed both in plasma and blood cells compartments. For instance, in the case of docetaxel and irinotecan the partition ratio between erythrocytes and plasma varied from 0.02 to 1.44 and from 0.7 to 2.8, respectively. For drugs prevalently distributed in plasma compartment, such as etoposide and nilotinib where the F_p is respectively 0.69 and 0.68, a simplified DBS-to-plasma conversion method (papers $n = 5$) was applied based solely on patient's Hct. In order to overtake the knowledge of both drug parameters such as $K_{BC/pla}$ or F_p and patients' characteristics such as the Hct value, several DBS-to-plasma conversion approaches were proposed directly based on empirical data: the simple application of a CF (paper $n = 6$) or regression analysis parameters (slope and intercept) (paper n = 6).

Finally, the direct correlation between DBS and plasma concentration was possible for drugs that were equally distributed between blood cells and plasma compartments (e.g., busulfan which is bound for the 4649% to blood cells) (paper $n = 7$). Clearly, for drugs that prevalently accumulate in blood cells compartment and thus the pharmacological target is already defined in whole blood, the direct correlation was exploited between DBS and whole blood concentrations: this was the case of everolimus.

It is worth to note that sometimes discrepancies can be observed in DBS-based methods developed for the quantification of the same drug. This is the case of imatinib: Antunes *et al.* (41) calculated an imatinib F_p of 0.81 in patients affected by CML, and for this reason Hct- and F_p-based normalization was applied. On the contrary, in Iacuzzi *et al.* (42) an imatinib F_p of 0.45 was calculated and thus the simplified Hct-based normalization was used. This divergence can be due to the different set of patients affected by different pathologies or to the different conversion approaches used.

In some papers, more than one DBS-to-plasma conversion approach was proposed for the same DBS-based method. In general, this is useful to find simpler correlation methods (i.e., those based on CF or regression analysis) that allow to avoid the use of values that need to be calculated, such as Hct, F_p and $K_{BC/pla}$. DBS-to-plasma conversion methods based on a CF or regression analysis often allowed to obtain the best predictive performance. Anyway, these normalization approaches require awareness when the DBS-based method needs to be translated to a more extended or different sample population since they strictly depend on patients set characteristics used to perform the analysis.

Ideally, the DBS-to-plasma conversion method applied should be further validated with an independent set of patients' samples, consequently doubling the sample size. This is particularly important for the conversion methods based on a CF or regression analysis. According to the guidelines, in order to make a cross-validation between DBS and plasma concentrations, the sample size should be at least of 40/50 samples (80 samples in total to further validate the conversion method). From the performed literature research, only in 48% of the articles (10 out of 21) at least 40 samples are analyzed and only in 1 paper (42) the CF found was applied to an independent set of patients ($n = 12$). Nonetheless, the relatively low disease incidence justifies the difficulty to obtain patients' samples and thus the small sample size.

DBS is a promising tool for TDM and may be even more convenient than plasma for specific drugs, in particular those highly partitioned in blood cells. From this review, we can conclude that, in most studies, DBS measurements, after proper conversion, can replace plasma analysis without compromising the reliability of the obtained data. Consequently, DBS can be use as alternative method to the standard venous blood sampling, with evident advantages for both patients and pre-analytical aspects.

In general, we can affirm that, when a DBS-plasma correlation method is needed, speculations can be made on a theoretical basis, but then it is useful to try different approaches in order to find the method that correlates, as accurately as possible, the values found in DBS with those obtained with the reference method.

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AUTHORS CONTRIBUTIONS

Valentina Iacuzzi: Conceptualization, Investigation, Data curation, Writing - original draft preparation; Bianca Posocco: Conceptualization, Investigation, Data curation, Writing - original draft preparation; Martina Zanchetta: Investigation, Data curation; Sara Gagno: Investigation; Ariana Soledad Poetto: Investigation; Michela Guardascione: Resources; Giuseppe Toffoli: Supervision, Writing – Review & Editing.

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