Journal of Pharmaceutical and Biomedical Analysis 130 (2016) 231–243

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Clinical feasibility of dried blood spots: Analytics, validation, and applications

Yeliz Enderle [1], Kathrin Foerster [1], Jürgen Burhenne *

Department of Clinical Pharmacology and Pharmacoepidemiology, Heidelberg University Hospital, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form 17 June 2016
Accepted 18 June 2016
Available online 23 June 2016

Keywords:
Dried blood spots (DBS)
Analytical validation
Clinical validation
Clinical applications

ABSTRACT

Dried blood spots (DBS) sampling and their specific advantages are becoming common in analytical and clinical routine. Being first established for metabolic disorder screening in neonates, its use emerged to a broad spectrum of clinical applications. Although DBS are easily generated, the conduction of specific analytical and clinical validation procedures should be obligatory when implementing DBS for clinical purposes, e.g. therapeutic drug monitoring or clinical drug trials. A respective recommendation has already been published by the European Bioanalysis Forum. Since no official guidelines are present, investigators are currently free in DBS procedure development and validation. This review summarizes and discusses published clinical validation procedures in relation to their applications to highlight the clinical feasibility of DBS.

1. Introduction

1.1. Principle of blood sampling

For a long time, whole blood was reputed as a complex matrix, complicating the sample preparation, sample management, and analysis [2]. Erythrocytes' rupture causes hemolytic matrix, which significantly affects the interpretation of analytical results [3]. Thereafter, blood plasma instead of whole blood was considered to be the matrix of choice to quantify therapeutic drug concentrations in patients. Since then, debates have been ongoing, whether whole blood would suit better for assessing drug concentrations [1].

Nowadays, the increasing affordability of sensible LC–MS/MS techniques enables precise drug quantification in matrices incorporating even vast interfering compounds [3]. Former analytical challenges with whole blood are now at a minimum. In 1963, Guthrie and Susi implemented the use of dried blood spot (DBS) as a sampling technique for whole blood [4]. This set the foundation for alternative drug exposure measurements. DBS, easily generated by heel- or finger-pricking, require only a few droplets of blood and after brief instructions patients are skilled to create DBS at home by themselves [1]. To confirm reproducible paper spots, Good Blood-Spotting Practices (GBSP) were established by the European Bioanalysis Forum (EBF) in 2011 [5]. Apart from basic conditions, e.g. disinfection prior to pricking and discarding the first blood drop because of tissue fluid, a perfect spot fills out the sketched filter paper circle. The freshly prepared DBS drop should be dried sufficiently at room temperature before transportation to laboratory [6].

1.2. Advantages of DBS

For in vivo drug concentration assessments, several advantages are presented with DBS. In contrast to common venipuncture, DBS sampling is minimal-invasive, rather less painful, and smaller amounts of blood are necessary. As a consequence, blood collection from children, even from neonates [1], as well as from patients suffering from phlebitis [7] is no longer a limitation. Furthermore, using DBS contributes enormously to the European directive on the protection of animals used for scientific purposes [8]. With DBS, blood can be easily sampled through tail vein, which promotes an animal-friendly preclinical drug development [9]. DBS enable patients to sample the analytical specimen at home at any required time. Especially, for therapeutic drug monitoring (TDM) this is beneficial, because it simplifies sampling at trough level [6]. DBS are robust to be shipped, can be stored at ambient temperature, and are able to be sent by mail without taking special precautions [10]. In contrast, blood samples need to be centrifuged for plasma generation and should be transported under chilled conditions. Establishing and obtaining standards for plasma handling

* Corresponding author.
E-mail address: juergen.burhenne@med.uni-heidelberg.de (J. Burhenne).
[1] Both authors contributed equally.

http://dx.doi.org/10.1016/j.jpba.2016.06.026
0731-7085/

in resource-limited settings are often challenging and even impossible [11]. Certain compounds are not stable in fresh blood, e.g. due to enzymatic degradation [12]. DBS offer a strategy to circumvent drug degradation, because the degradation causing enzymes denature when being dried on filter paper [13]. Also in case of labile prodrugs, glucuronides, metabolites, or photosensitive drugs, DBS are a helpful tool [3]. Finally, DBS diminish the infectious risk for laboratory personal when preparing blood samples of unknown – potentially HIV, hepatitis or other blood-borne viruses – origin [12,14].

1.3. Biomedical applications of DBS

The first large scale clinical DBS application was the neonatal screening on phenylketonuria [4]. Its spectrum is continuously rising. During drug development it is utilized in preclinical and clinical trials [15]. The advantages for forensic toxicology [12], doping, and environmental contamination [16] are obvious and the use of DBS has emerged to clinical pharmacology within TDM and epidemiologic disease surveillance [17]. Furthermore, DBS application has been tested promising for molecular techniques (e.g. immunoassays) [18], genotyping [19], and several RNA/DNA assays [20–22].

1.4. Challenges of DBS

Despite the described advantages, DBS still offer analytical challenges. The analysis of a single drop of blood requires sensitive and expensive analytical techniques. Often only one spot per patient is available and no test replications are possible. Patients need to be trained before doing sampling on their own. But even with adequate training, spots sometimes display a non-suitable shape for analytics. Further on, spot contamination risk arises if the same person doses the drug and prepares the DBS [1]. As mentioned before, drug concentrations are usually quantified in plasma and whole blood. An additional validation becomes necessary, if a DBS assay should be implemented. DBS sampling commonly covers capillary blood, which is a mixture of arterial and venous whole blood plus interstitial fluid. Physiologically, it differs from venous whole blood [2,23]. The drug concentration discrepancies between venous whole blood or plasma and DBS capillary blood need to be assessed and make a clinical validation obligatory [5].

1.5. Clinical feasibility

This review discusses the clinical feasibility of DBS with special aspect on patients ability to adapt to DBS sampling in future. We searched the PubMed database on terms: "dried blood spot(s), paper spot(s), blood spot(s), filter paper, dried spot(s)" AND "clinical trial". All published method developments and applications with clinical validations until March 2016 were evaluated and are summarized in Table 1. The aspects on laboratory and clinical validation, which were considered by the authors are listed.

2. Analysis and validation of dried blood spots

The validation of the special requirements for quantitative DBS methods is currently not covered by official guidelines, since the quantitative implementation into clinical routine is just developing in the last four to five years. The analytical validation of a quantitative DBS method should be conducted in the first step according to the FDA and EMA guidelines [24,25] as a basis for good laboratory standards and because of the common consensus for these guidelines to the global harmonization on method validation. Further considerations on matrix and sampling specific validation parameters are based on expert knowledge, especially on the in 2011 published "EBF recommendation on the validation of bioanalytical methods for dried blood spots" [5]. The European Bioanalysis Forum (EBF) is a nonprofit organization and a source of broad bioanalytical network, which discusses general topics of bioanalytical interest, e.g. scientific, regulatory, as well as technological issues. Hence, the DBS methodology is not yet accepted by the EMA for drug development studies and the FDA expects the DBS concentrations being the same as from conventional techniques [26]. The complete validation including the analytical part and especially the clinical validation of quantitative DBS methods is still mandatory [27], as long as the sampling technique itself is not implemented in the development and approval process of the certain compound [28].

2.1. Analytical validation of quantitative dried blood spot methods

Based on the FDA guideline "Guidance for Industry – Bioanalytical Method Validation" [24], the general validation of any bioanalytical drug quantification method comprises selectivity, accuracy, precision, recovery, calibration, standard curve, and stability and should also be applied to any DBS method development. Selectivity has to be tested to determine the ability of the developed method to quantify the substance of interest in the presence of possible other components within the sample, such as endogenous matrix specific compounds, metabolites, or degradation products, as well as possible co-medication of the patient. Accuracy represents the truth of the measured concentration compared to the nominal concentration of each calibration or quality control sample, being within 15% of the nominal concentration and 20% at the lower level of quantification (LLOQ). Precision describes the spread of replicate measured results and is defined by the standard deviation of mean test results. The range should be within 15% of the coefficient of variation (20% at the level of LLOQ) and examined for within-batch and batch-to-batch values. Recovery shows the extraction efficiency of the developed method. The parameter should be consistent, reproducible, and precise for the drug and the respective internal standard. The standard calibration curve as the basis for the quantification of unknown samples should be generated in the same biological matrix, which means that it should be spotted onto filter paper. At least six concentration levels per standard curve, depending on the expected concentration range of the study samples including the LLOQ have to be generated. Stability as a general validation parameter has to be investigated for the analyte within the matrix, the storage conditions, and the analysis system. These parameters can be covered by investigating freeze and thaw stability, short- and long-term stability, stock solution stability, and the post-preparative stability.

2.2. DBS specific validation parameters

The DBS validation is influenced by several issues not covered by the FDA or EMA guidelines. Some parameters (e.g. stability, recovery) have to be adjusted for DBS procedures whereas other parameters (e.g. hematocrit) are exclusively applicable only to DBS sampling [5,29]. Considerations on the use of capillary whole blood instead of plasma as sampling matrix comprise further validation investigations. Possible hemolysis of the used whole blood and the influence on the preparation of the calibration and QC samples should be investigated and classified [5].

Stability experiments should be adjusted specifically for DBS. The advantage of the ease of shipping conditions, in which usually no refrigerating is needed, leads to the need for broader stability experiments (temperature range, illumination) since shipping conditions are not fixed. Extreme values possible during shipping have been investigated by Bowen et al. and might be part of the

Table 1
Overview of published clinical validations with DBS.

Drug	Validation guidelines	DBS extraction	HTC tested	Stability tested	Chrom. tested	BSV tested	DBS blood	No. of samples	Participant	Calculation	Statistical analysis	Ref.
theophylline	n.n.	punch	no	no	no	no	capillary	21	patient	no	linear regression	[68]
HIV drugs	n.n.	punch	no	no	yes	no	venous (5 µL)	70	patient	no	linear regression	[69]
HIV drugs	FDA	n.n.	n.n.	n.n.	n.n.	n.n.	capillary	11	patient	no	linear regression	[7]
nevirapine efavirenz	CLSI FDA	punch	no	30 °C for 7 d RT for 7 months	yes	yes	capillary	80	patient	$c_{plasma} = \frac{c_{DBS}}{1-HTC} \times f_{bpp}$ f_{bpp} = fraction of protein bound analyte	linear regression Bland-Altman	[70] [121]
HIV drugs	CLSI FDA	punch	no	30 °C for 7 d RT for 7 months	yes	yes	capillary	50	patient	$c_{plasma} = \frac{c_{DBS}}{1-HTC} \times f_{bpp}$	Bland-Altman	[70] [71]
nevirapine	FDA EMA	punch (blood), whole spot (milk)	no	−80 °C, RT for 15 months	no	yes	capillary, breast milk (30 µL)	5	patient	$c_{plasma} = \frac{c_{DBS}}{1-HTC} \times f_{bpp}$	no	[72]
tenofovir emtric-itabine tenofovir-diphosphat	n.n.	punch	0.1–0.76	−80 °C, −20 °C, 4 °C, RT for 7 months	yes	yes	venous (25 µL)	8	patient	no	linear regression	[73] [74]
efavirenz	FDA	punch	no	−70 °C, 4 °C, RT, 37 °C, 45 °C for 1 month −20 °C for 1 year	no	no	venous (100 µL)	31	patient	no	Spearman correlation coefficient linear regression	[75]
efavirenz	FDA	punch	yes	RT, 40 °C for 24 h −40 °C for 12 months	yes	no	venous (50 µL)	46	patient	no	Deming regression Bland-Altman	[11]
imatinib nilotinib dasatinib	FDA	punch	0.3–0.6	−20 °C, RT for 28 d (dasatinib 40 °C for 3 d)	no	no	venous (10 µL)	18, 2, 3	patient	$c_{plasma} = \frac{c_{DBS}}{1-HTC}$	Bland-Altman	[76]
pazopanib	n.n.	punch	0.2–0.65	ambient temperature for 75 d	no	no	venous capillary (15 µL each)	12	patient	$c_{plasma} = \frac{c_{DBS}}{1-HTC}$	Passing-Bablok Bland-Altman	[41]
etoposide	FDA	whole spot	0.3–0.6	−20 °C for 7 d 40 °C for 28 d	no	no	venous (20 µL)	28	patient	$c_{plasma} = \frac{c_{DBS}}{1-HTC}$	Deming regression Bland-Altman	[78]
tamoxifen endoxifen	FDA EMA CLSI	punch	0.2–0.48	RT for 4 months 2–8 °C, 37 °C for 24 h	yes	yes	capillary	44	patient	$c_{serum} = \frac{c_{blood}}{(1-HTC)+K_{BC.Serum}+HTC}$	Deming-regression Bland-Altman	[32] [40]
tamoxifen endoxifen further metabo-lites	EMA	punch	0.25–0.5	−20 °C, 25 °C, 45 °C for 20 d	no	no	capillary	91	patient	$c_{plasma} = \frac{c_{DBS}}{1-HTC} \times CF$	Passing-Bablok Spearman correlation Bland-Altman	[79] [80]
ciclosporine A tacrolimus	FDA	punch	no	RT for 14 d	no	no	venous (25 µL)	150, 150	patient	no	Passing-Bablok Bland-Altman	[81]

Table 1 (*Continued*)

Drug	Validation guidelines	DBS extraction	HTC tested	Stability tested	Chrom. tested	BSV tested	DBS blood	No. of samples	Participant	Calculation	Statistical analysis	Ref.
ciclosporine A tacrolismus sirolimus	n.n.	punch	no	−20 °C, 4 °C, 25 °C for 30 d 37 °C for 5 d	yes	yes	venous (50 µL)	79, 115, 68	patient	no	linear regression	[83]
ciclosporine A tacrolimus sirolimus everolimus	FDA	punch	0.2–0.5	−80 °C, 22 °C, 37 °C for 28 d	no	yes	venous (50 µL)	57, 50, 36, 55	patient	no	Passing-Bablok	[84]
tacrolimus	FDA	punch	0.2–0.48	−20 °C for 35 d 4 °C, 24 °C for 10 d 50 °C for 24 h	yes	yes	venous (30 µL)	50	patient	no	n.n.	[87]
tacrolimus	n.n.	punch	no	RT for 4 weeks	no	no	capillary	18	patient	no	linear regression Bland-Altman	[85]
ciclosporine A	FDA	punch	0.2–0.72	Ambient temperature for 17 d 4 °C for 45 d	no	no	capillary	38	patient	no	Deming regression Bland-Altman	[88] [89]
mycophenolic- acid	n.n.	punch	no	RT, 4 °C for 20 d	no	no	capillary	77	patient	$c_{plasma} = \frac{c_{DBS}}{1-HTC}$	Passing-Bablok Bland-Altman	[90]
voriconazole posaconzale fluconazole	FDA	punch	0.2–0.45	−80 °C, RT, 37 °C, 50 °C for 12 d	no	yes	capillary & venous (50 µL)	10, 7, 11	patient	CF calculated	Passing-Bablok Bland-Altman	[91]
pregabalin	EMA	punch	0.2–0.6	2–8 °C for 4 months	yes	no	venous (50 µL)	12	patient	equation established	Bland-Altman	[94]
valproic acid	n.n.	punch	0.25–0.5	45 °C for 21 d	no	no	capillary	17	patient	CF calculated	n.n	[95]
carbamazepine valproic acid phenytoin	n.n.	punch	no	−20 °C, 25 °C for 10 d	no	no	venous (2 drops)	101, 92, 49	patient	$c_{plasma} = \frac{c_{DBS}}{(1-HTC\times(1-K))}$ K = red blood cell-to-plasma portioning,	Deming-regression Bland-Altman	[67]
proguanil cycloguanil	n.n.	punch	no	−20 °C, 4 °C, 20 °C, 50 °C for 20 d	no	no	capillary	10	healthy volunteer	no	Bland-Altman	[96]
clozapine	USP guidelines	whole spot	no	RT for 50 d	no	no	venous (25 µL)	30	patient	CF calculated	Bland-Altman	[97]
amitriptyline nortriptyline clomipramine & active metabolites	FDA	punch	0.2–0.5	−20 °C for 30 d 2–8 °C, RT for 90 d	yes	yes	capillary	4, 11, 9	patient	no	linear regression	[98]
rifampicin	ICH	punch	no	RT for 9 h	no	no	capillary	5	patient	no	linear regression	[102]
moxifloxacin	FDA	punch	0.2–0.5	−80 °C, RT, 50 °C for 4 weeks	no	yes	capillary	6	patient	no	linear regression Passing-Bablok regression	[103]

rifampicin clar-ithromycin & metabolites	FDA	punch	0.2–0.5	37 °C for 30 d ambient temperature for 60 d 50 °C for 15 d	no	yes	capillary	12	patient	no	linear regression Deming regression	[104]
miltefosine	FDA EMA EBF	punch	0.1–0.51	−70 °C, 20 °C, RT, 37 °C for 162 d	yes	yes	capillary	16	patient	CF calculated	Deming regression Bland-Altman	[105]
metformine sitagliptine creatinine	n.n.	whole spot	no	RT for 4 weeks	no	no	capillary (40 μL)	2	patient	n.n.	n.n	[106]
tadalafil sildenafil ambrisentan bosentan	FDA EMA EBF	punch	0.25–0.6	RT, direct sunlight for 147 d −20 °C, 37 °C for 14 d	no	no	capillary	29, 53, 27, 55	patient	CF calculated	Deming regression Bland-Altman	[45]
topiramate	n.n.	punch	no	RT for 1 week	no	no	n.n.	21	patient	no	Bland-Altman	[109]
phenobarbital	n.n.	punch	no	−20 °C, 4 °C, RT for 1 month	no	no	venous (20 μL)	50	patient	$c_{plasma} = c_{DBS} \times \frac{100}{(100-HTC)}$	linear regression Bland-Altman	[110]
rufinamide	n.n.	punch	no	−20 °C, 4 °C, RT, for 30 d	no	no	n.n.	14	patient	$c_{plasma} = c_{DBS} \times \frac{100}{(100-HTC)}$	linear regression	[111]
phenytoin	ICH	punch	no	−20 °C, 4 °C, RT, 37 °C for 4 weeks	yes	no	n.n.	17	patient	equation established	linear regression	[112]
sirolimus tacrolimus	n.n.	punch	no	−20 °C, 4 °C, 25 °C for 30 d 37 °C, 60 °C for 5 d	yes	yes	capillary	34	patient	no	Deming regression Bland-Altman	[83] [101]
lopinavir ritonavir	FDA	punch	no	−20 °C for 20 d 4 °C for 24 h	no	no	venous (25 μL)	19	patient	no	linear regression Bland-Altman	[113]
linezolid	FDA	punch	no	−20 °C, 4 °C, RT, 37 °C for 1 month	yes	no	n.n.	9	patient	$c_{plasma} = c_{DBS} \times \frac{100}{(100-HTC)}$	Bland-Altman	[114]
propranolol	n.n.	punch	no	−20 °C, 4 °C, RT for 1 month	no	no	venous	7	patient	$c_{plasma} = c_{DBS} \times \frac{100}{(100-HTC)}$	n.n.	[115]
acet-aminophen	FDA CLSI	punch (manual extraction), whole spot (automatic)	no	−20 °C, 4 °C for 1 week −80 °C for 1 month ambient temperature for 24 h	no	no	n.n.	n.n.	n.n.	n.n.	Deming regression Passing-Bablok Bland-Altman	[108]
methotrexate & poly-glutamates	FDA	punch	0.3–0.55	−80 °C, RT for 2 months	no	no	capillary	47	patient	CF calculated	linear regression Bland-Altman	[116]
midazolam & 1-OH-midazolam	FDA	punch	no	RT for 4 weeks	no	no	capillary (20 μL)	12	healthy volunteer	no	linear regression	[19]
fluriprofen & 4-OH-fluriprofen	SFSTP	punch	no	−20 °C, 4 °C, ambient temperature, 40 °C for 1 month	no	no	capillary	10	healthy volunteer	no	linear regression	[119]

Table 1 (*Continued*)

Drug	Validation guidelines	DBS extraction	HTC tested	Stability tested	Chrom. tested	BSV tested	DBS blood	No. of samples	Participant	Calculation	Statistical analysis	Ref.
MK-1775	FDA	punch	0.16–0.85	−20 °C for 6 months ambient temperature for 14 months 40 °C for 8 d	yes	yes	venous (40 µL)	12	patient	no	linear regression	[120]
ceftriaxone	FDA	punch	0.31–0.67	−80 °C, −20 °C, 4 °C for 6 months 21 °C, 35 °C for 9 weeks	yes	yes	capillary & venous	10	healthy volunteer	no	Spearmans-Rank correlation coefficient linear regression Bland-Altman	[66]

d = days; n.n. = not named; no = not considered; yes = considered; HTC = hematocrit; chrom. = chromatographic; BSV = blood spot volume; no. = number; Ref = Reference.

validation [30]. Regarding DBS storage conditions closer attention need to be dedicated on sampling site, e.g. patients' home, and – on an international basis – diverse climate conditions concerning sun light exposure, temperature, and humidity [31]. The EMA guideline requirements already define matrix effect experiments quite well. The effect is supposed to be tested equally for the drugs and their corresponding internal standards. Beside the blood matrix, additionally extracted cellulose materials from the DBS itself, may contribute to an enhanced matrix effect [32]. Hence the matrix effect has to be tested for each filter paper in combination with the respective analyte. The matrix effect validation experiments compare the mean peak area response of fully cut blank DBS spiked with a known concentration after extraction to the respective matrix-free LC eluent containing 100% of standards and internal standard [45]. Because internal standards are mainly added to the extraction solution and not directly onto the DBS, matrix effect experiments may differ between analytes and their internal standards. Commonly, internal standards are added prior to the extraction to compensate for recovery variations. Considering DBS this is not applicable and the current practice comprises the addition of internal standards within the extraction solvent [5,33]. The EBF consortium performed experiments investigated this influence and revealed that internal standard addition with the extraction solvent is not applicable for any tested drug [33]. But in contrast, the only feasible internal standard application prior to the extraction procedure is by the use of the CAMAG spray device to add the internal standards homogenously on top of the already dried blood spots [33,34]. Further investigations of Abu-Rabie et al. on the internal standard addition to delete hematocrit and recovery bias also revealed the CAMAG spray device as the best feasible application method [35]. Therefore, the intended way of internal standard addition should be part of validation procedures and investigated for each individual drug and clinical implementation.

Based on the recommendations of the EBF in 2011, the extraction recovery of DBS methods has to be evaluated more thoroughly than required by the FDA for plasma validation [5]. The influences of long-term storage e.g. by aging of DBS [36,37], the issue on internal standard application to the extraction procedure [35], and different hematocrit levels [37] in combination with drug concentration ranges [38] should be investigated. Varying extraction efficiency in a certain DBS procedure directly leads to varying results and therefore long-term stability tests ideally involve concurrent recovery experiments [32,37]. Abu-Rabie et al. revealed extraction efficiencies being above 90% are not biased greatly by hematocrit [35]. When combining internal standard addition as a spray onto the DBS and performing a whole spot analysis, the elimination of hematocrit based recovery bias can be achieved [35]. Although, discussed by Koster et al. the analysis of whole DBS does not compensate hematocrit based recovery bias when influenced in combination with the drug concentrations [38]. The recovery performance within DBS analysis is a complex issue and should be tested carefully.

The amount of blood spotted onto a paper card does not seem to have a great influence on the quantitative outcome of an analyzed aliquot punch. The blood spot should be evaluated concerning its size and form. Different filter paper types need different amount of blood to generate identical spot sizes. This becomes especially important, when using larger punch diameter (min. 6 mm) to obtain higher sensitivity or to reduce spot in-homogeneities [5,39]. The EBF recommends validating a certain range of blood volume spotted, as well as the ±50% threshold values of the expected blood volume. Furthermore, the DBS has to be visually inspected in considerations of a thoroughly soaked, not blurred blood spot to ensure a completely soaked aliquot punch for the quantification. DBS inhomogeneity did not seem to influence the analysis much [5]. The EBF consortium published new results that hematocrit, chemical characteristics of the certain drug and especially card type reveal

influence spot homogeneity [39]. Extreme values of hematocrit levels (20% and 70%) in combination with a very small 1 mm punch diameter, taken from the center and the perimeters of the spot were tested. The findings support the necessity of validating each individual drug in combination with a fixed card type.

Hematocrit is the most challenging issue concerning the development of a DBS quantification method. It has a main impact on the blood distribution on the filter card, due to the specific blood viscosity. This influences the spot size and therefore directly the quantitative result when using an aliquot punch. The EBF DBS consortium investigated a hematocrit range from 20% to 70%, revealing a major impact on the spot formation, accuracy and precision, but especially on the recovery for freshly spotted DBS and also for aged DBS [36]. The variations are drug dependent and often beyond the acceptable validation ranges of 15%. Spotting an accurate volume and analyzing the whole spot eliminates the hematocrit effect at all, but limits the intended simple applicability of the DBS sampling technique, as discussed before. Several equations have been published to correct for hematocrit values [40,41], by using the normal hematocrit value for women and men or the individual parameter of the patient [42]. Capiau et al. published a method for measuring the individual hematocrit value in a separate DBS. K^+ concentration levels in DBS extracts from 3 mm punches were determined by indirect potentiometry and used to calculate the hematocrit value and generate a linear calibration for the hematocrit range of 19–63% [43]. First evidence reveals that drug concentrations have to be considered in combination with the certain hematocrit level, because the hematocrit effect appears not to be linear and accounts for varying extraction recoveries [38]. QC samples covering the concentration range at different hematocrit levels should be included in the validation process to quantify the hematocrit influence [44,45].

2.3. Different sampling devices

Different micro sampling devices for capillary whole blood and plasma have been recently developed and investigated to overcome or at least minimize the pitfalls of conventional DBS sampling. The most influencing parameter is still the variability of individual hematocrit levels and their impact on the spot size and formation, but also the applicability for the patients, the storage, and cross contamination issues. Furthermore, most therapeutic ranges for TDM are usually referred to plasma concentration. The conversion factors for calculating the DBS results in plasma concentrations are usually unknown, moreover difficult to determine. To prevent any hematocrit effect at all, to overcome matrix effect related issues due to whole blood samples and to include analytes where the "red blood cells/plasma" partitioning is not well explored, devices for microsampling of plasma have been developed. The sampling of capillary whole blood in a capillary, followed by centrifugation and displaying the plasma onto a filter paper card is one way to generate plasma samples by a fingerpick [46,47]. This application replaces the intended use of DBS, but medical staff and laboratory equipment such as centrifuges is needed again. These issues have been overcome by developing a plasma extraction card (Noviplex™) which separates the blood cells out of a capillary whole blood drop by filtration and generating a dried plasma sample [48]. Another way to eliminate the hematocrit effect when sampling capillary whole blood can be achieved by using a known blood volume and analyzing the whole DBS. Li et al. and Youhnovski et al. discussed respectively perforated DBS (PDBS) pre-cut DBS (PCDBS) to investigate the applicability and the impact of different hematocrit levels on the quantification of these spots [49,50]. Both applied accurate blood volumes for method validation and showed that the hematocrit effect can be eliminated by this device in a regulated environment where volumetric blood sampling is possible. A further development by Meesters et al. led to the microsampling device of DMPD (dried matrix on paper disks) [51] and includes a special cartridge for storage and shipping of fixed sized DBS, which are also generated with an accurate blood volume by using capillaries or pipettes. In order to maintain the benefits of the DBS sampling technique without using volumetric devices several approaches have been made. One method to generate exact volume samples is to limit the available filter paper matrix respectively the absorptive capacity of the available filter paper. HemaSpot™ is a DBS device including a fan shaped filter paper called HemaForm™ [52,53]. The device includes the fan shaped filter paper (8 slices). A desiccant disk covered by an application disk and has to be loaded with two drops of capillary whole blood [54]. First evidence reveals a more consistent recovery within a hematocrit range of 25–65%, but the device needs to be further investigated to examine the blood volume per slice independent of the volume of dropped blood [55]. Polley et al. published a shaped filter paper collection device allowing a capillary blood sampling of 21.1 μL for the screening of Plasmodium falciparum infected blood [56]. The laser cut filter paper has to be further investigated on the impact of different hematocrit levels, if it is intended to use for drug quantification. Another approach to limit the blood volume soaked by the sampling matrix is the Volumetric Absorptive Microsampling (VAMS™). The device consists of a removable polymeric tip placed on a plastic pin. The commercially available device called Mitra™ Microsampling device [57] absorbs 10 μL of capillary whole blood with a volume variation of 5% within a hematocrit range of 20–70% tested with caffeine [10]. A published quantitative method for emixustat using this device revealed acceptable accuracy and precision parameters within a hematocrit range of 30–55% [58]. The device should be further investigated and validated for each drug [10]. The development of a holding cartridge for commercially available DBS filter paper cards allows the microfluidic-based accurate sampling of 5 or 10 μL of capillary whole blood [59]. Four sized capillaries get filled with capillary whole blood, when attached to the blood drop. After closing the device, the filter paper card gets in contact with the sampled blood at the outlet of the capillaries creating 4 DBS with an accurate blood volume. The reproducibility of the sampled blood volume has been shown for caffeine and within a hematocrit range of 26–62% [59]. The closed device also serves as a protection against cross contamination and for storage and shipping. Another sampling device consists of a Whatman 903 filter paper layer and a microfluidic layer on top with an inlet, a capillary, and an outlet [60]. Once a capillary blood drop is disposed on the inlet, the sized capillary fills with an accurate volume of blood and breaks through a dissolving film at the outlet generating an exact DBS. The accuracy of blood volume per DBS was tested with amphetamine and revealed an amount of 6.1 μL capillary whole blood with a variation of 8.8%. Further investigations on the impact of different hematocrit levels and blood temperature on the dissolving time of the thin dissolving film have to be performed [60]. The latest approach is the so called hemaPEN™ which combines the benefits of accurate capillary blood sampling and the simple application procedure for patients [61]. The device is constructed like a retractable pen, able to collect and store four DBS with an accurate volume within the device. The ongoing developments of new approaches and sampling devices reflect the great possibilities but also the challenges of the microsampling technique and its future within the field of bioanalytical research and clinical drug monitoring.

2.4. Clinical validation of quantitative dried blood spot methods

The clinical validation of developed DBS methods is still mandatory in order to be able to implement and compare the results of DBS e.g. with plasma concentrations [27]. Rowland and Emmons developed at an early stage of DBS method application a decision

tree as when to use DBS or plasma quantification methods [23]. The blood cell partitioning and the protein binding behavior, e.g. the unbound fraction in plasma, are the parameters influencing the correlation between plasma and DBS concentrations. Two situations have to be considered generally when a DBS sampling and quantification method is supposed to be chosen: First, if the blood cell partitioning is not constant, and second, when the erythrocyte binding is temperature dependent, e.g. cyclosporine and tacrolimus [23]. Therefore, and for the above discussed analytical challenges, bridging studies to evaluate the relationship or agreement of the DBS and plasma concentrations need to be performed and statistically analyzed [27]. At present, there is no official guideline defining the requirements for a cross validation between DBS and plasma concentrations. Two guidelines can be considered partially to correlate the concentrations of DBS and plasma samples. The EMA guideline on bioanalytical method validation comprises a section on cross validation, meaning the comparison of two different methods. The guideline recommends that the difference between the two sample values has to be within 20% of the mean for ≥67% of the samples [25]. Furthermore, the CLSI guideline for "Method Comparison and Bias Estimation Using Patient Samples" [62] provides a complete course of action from designing and analyzing a method comparison study, based on a reference method and therefore using linear regression analysis. This guideline recommends using at least 40 sample pairs as basis for statistical analysis. When combining these two guidelines, a regression has to be performed to investigate the correlation factor for the DBS versus the plasma concentration and Bland-Altman difference plots to examine the agreement between the results of the two quantification methods. If considering the current routine method as reference method, a linear regression can be performed [62]. Although most commonly a Deming or a Passing-Bablok regression is applied (rather than using a least-squares linear regression) to observe DBS and plasma concentrations measured as error-prone variables due to their analytical nature [63–65]. Bland-Altman difference plots performed by comparing DBS versus plasma concentrations show a notable difference when the correlation factor of the two quantification methods is unequal one. Therefore, these plots should be performed by using the measured versus the DBS based calculated plasma concentration [29,66].

3. Application of DBS

3.1. Drugs with a narrow therapeutic index

If a drug is characterized by a narrow therapeutic margin, it is recommended to conduct a TDM on a regular basis. DBS is a powerful tool facilitating these recurring pharmacokinetic measurements. Patients are not required to consult their physician for blood withdrawal [67]. By a simple finger prick, a drop of blood is easily put onto a filter paper at home. During the past decade, several research groups considered and proofed this option for a remarkable variety of drugs. One of the first studies was done by Watson in 2001 to quantify theophylline [68]. He designed a study to observe a positive correlation between standard venous blood donation and capillary whole blood (DBS). Duplicates at each time point and participant were taken. Good blood spotting practices were taken into consideration. It was stated that an even better correlation must be realized by better sampling performance. Blood spots need to be saturated completely or a smaller diameter should be cut at the center of the spot to get comparable samples [68].

3.1.1. Antiretroviral therapy

The effective HAART-therapy strongly relies on patient's compliance to combat HIV effectively. Strengthening compliance lessens the emerging resistance formation, adverse and toxic effects. Plasma concentrations of protease inhibitors (PI) and non-nucleoside transcriptase inhibitors (NNRTI) correlate well with pharmacological effects. Laboriously, plasma probes need to be treated before sample preparation to deactivate the infectious virus. Koal et al. investigated the use of DBS to circumvent this compelling aspect [69]. Another HIV-TDM method on darunavir, etravirine, raltegravir, and ritonavir was published in 2011 with exclusively male participants [7]. Kromdijk et al. quantified nevirapine and efavirenz in DBS. DBS results were converted into theoretical plasma concentrations with a formula (see Table 1) and then correlated with plasma values present. It was demonstrated, that comparable results were gained whether mean (45%) or individual hematocrit values were used for calculation. Utilizing mean values makes routine analysis simpler, because the identification of individual hematocrit is negligible [70]. Suitability was tested, when DBS were sampled by patients at home [71]. Patients were trained and subsequently asked on "practical capability" with the help of a questionnaire. 51% preferred DBS and the authors stated that patients were capable to do home-sampling with a proper training beforehand. Another aspect for TDM justification is that it is especially important for critical subgroups of patients (e.g. children, pregnant women, poly-pharmacy regimes). Olangunju et al. focused on nevirapine quantification in DBS and dried breast milk spots. DBS were used to avoid ethical aspects when measuring infants' exposure to nevirapine during breastfeeding. Children were not even exposed to blood withdrawal [72]. Tenofovir and emtricitabine (NRTI) are nucleoside analogues, which are activated to its phosphorylated form in cells. Castillo-Mancilla et al. assumed DBS to be a more suitable matrix for blood concentrations measurements [73,74]. The use of DBS to accomplish clinical trials in resource-limited areas was investigated for efavirenz by Hoffman et al. [75]. Authors stated that hematocrit values between 35 and 48% have no impact on measurements. Amara et al. further investigated the use of DBS to overcome TDM obstacles in resource-limited settings. In this trial, lower hematocrit values were investigated, because HIV is often co-present with tuberculosis in these areas [11].

3.1.2. Cancer therapy

Considering cancer chemotherapy, high intra-individual variability is expected and TDM becomes interesting to accomplish the best personal efficacy and safety profiles. Outlying plasma concentrations can be lethal, leading to either toxic effects (e.g. neutropenia) or minor tumor cytotoxicity. The gentle sampling method of DBS suits perfectly for often anemic and weakened patients.

Kralj et al. compared blood to plasma concentrations of the three tyrosine kinase inhibitors imatinib, nilotinib, and dasatinib [76]. DBS sampling by finger prick was disregarded and DBS were created from venous blood, because the authors supposed no distinction as it was stated by Mohammed et al. [77].

Another tyrosine kinase inhibitor pazopanib was studied by de Wit et al. [41]. Authors discovered differences between DBS derived and observed plasma concentrations. The formula used for calculating the respective plasma concentration out of DBS measurements assumes that the drug stays in plasma entirely. Other scientists advice to incorporate drug specific plasma protein fraction for calculation [13]. DBS sampling was performed by a study nurse with a fixed volume spotted and related to other studies showing that there were no significant differences between patients' or professionals' DBS [71].

Rezonja Kukec et al. tested etoposide for DBS validity [78]. DBS were generated 3, 6, and 24 h after etoposide exposure. For extraction the complete spot was cut out. This is rather laborious but enables to disregard hematocrit values. To prevent recurrences in breast cancer therapy Jager et al. presented a TDM method for

tamoxifen and its metabolite endoxifen [32,40]. Again it was recommended to conduct proper training of patients to get usable capillary blood spots at an optimum. Antunes et al. evaluated the use of DBS for TDM of tamoxifen further [79]. It was shown that female's therapy regime should perfectly fit to her CYP2D6 activity status. The metabolic ratio of tamoxifen/endoxifen was measured as a surrogate for CYP2D6 activity. Importantly, the influence of co-medication on tamoxifen exposure was also investigated [80].

3.1.3. Immunosuppressive therapy

It is pivotal for patients' safety to monitor immunosuppressant drugs on a regular basis. Immunosuppressants easily accumulate into red blood cells. Measuring blood instead of plasma concentrations is standard to get robust results [23]. Sampling blood at correct times is important, because only trough levels are considered reliable for TDM. To substitute venous blood sampling with DBS would overcome wearisome consultations and improve patient's quality of life enormously.

Hinchliffe et al. investigated DBS application with ciclosporin A and tacrolimus [81]. DBS were prepared from venous whole blood. Referring to previously demonstrated correlation between venous and capillary prepared DBS [82], the method was supposed to be suitable for TDM purposes at patients' home [81]. Including sirolimus, Sadilkova et al. performed another DBS study on immunosuppressive drugs [83]. Koster et al. analyzed tacrolimus, everolimus, sirolimus, and ciclosporin A. Also they explored the effect of different DBS cards types on analysis [84]. Interestingly, everolimus displayed varying recoveries at different concentrations. This was supposed to be due to its high affinity to build hydrogen bonds with cellulose DBS paper. Cyclosporine A exhibited chromatographic effects at extreme hematocrit values and drug concentrations and its accumulation into erythrocytes and leukocytes is saturated at higher concentrations. A TDM method for tacrolimus by Koop et al. particularly focused on adolescent to adult patients during method validation [85].

Commonly, immunosuppressant drug concentrations are assessed with immunoassays. Several research groups highlighted the phenomenon that immunoassays display constantly higher concentrations compared to LC–MS/MS [86,87]. An overestimation of drug concentrations in blood gives privilege to LC–MS/MS measured DBS, because with LC–MS/MS no cross-reactivity or other reasons for overestimation are expected [87]. Wilhelm et al. draw special attention to good blood spotting practices, when investigating DBS for TDM of cyclosporine A [88]. When arriving at the laboratory, DBS were precisely examined on complete saturation on both sides of the spot. Furthermore, it was demanded that patients need to be trained for DBS sampling to prevent blood smearing. Outliers present in the study were samples from patients with liver failure. It was postulated that further method development needs to done, to be able to measure patients with severe liver failure [88,89].

For monitoring of mycophenolic acid an AUC calculation is performed, normally. This multiple sampling procedure is challenging for patients. Arpini et al. stated that DBS are beneficial and investigated the use of DBS for blood concentration measurements [90].

3.1.4. Triazole antifungals

Apart from well-known individual pharmacokinetics, there are several other facts essential for TDM under triazole therapy. Voriconazole is known for its non-linear pharmacokinetic and posaconazole is often absorbed incompletely. Van der Elst et al. explored the use of DBS for voriconazole, fluconazole, and posaconazole [91]. For clinical validation DBS were created from venous whole blood (VDBS) and capillary blood (finger prick DBS) and compared to plasma concentrations. Patient's satisfaction on duration, easiness to perform, pain registered during sampling, and patients' preferred method was assessed. Comprising, patients were satisfied with DBS sampling. 20 patients completed a questionnaire and 12 preferred the DBS method (60%). Pain when doing finger pricking was examined to be negligible. The questionnaire was established on the basis of conventional quality of life tests [92,93].

3.1.5. Antiepileptic therapy

In a study investigating pregabalin on DBS purposes, spots were compared to dried plasma spots, both prepared from venous whole blood. To bypass hematocrit effects, an alternative method to establish a correction factor was presented: A known concentration was measured at different hematocrit values (20–60%). Deviations between measured and theoretical concentration at each HTC level were used to establish a linear equation. By entering patient's individual HTC value into the equation, each patient's own correction factor was calculated [94].

Rhoden et al. evaluated the use of DBS for TDM of valproic acid. DBS samples were collected at trough level and highly correlated with plasma concentrations [95]. Most patients received more than one drug for antiepileptic therapy. Therefore, Kong et al. developed a multi-method to determine carbamazepine, valproic acid, and phenytoin in DBS [67].

3.1.6. Other drugs

To prevent malaria treatment failure, Lejeune et al. evaluated the use of DBS to quantify proguanil, chloroquine, and their active metabolites. Correlation was proven and analytes were stable for up to 4 days at equatorial temperatures of +50 °C [96].

Patients suffering from schizophrenia are often treated with clozapine. Although patient's quality of life improves enormously under treatment, clozapine bears a dose-related risk toxicity. Therefore, Saracino et al. described a DBS method to measure drugs exposure and prevent toxic side effects [97].

Meanwhile, some guidelines advice to monitor amitriptyline, nortriptyline, clomipramine, and their active metabolites. Tricyclic antidepressants display a large variability in terms of clearances and exhibit small therapeutic windows. To facilitate TDM Berm et al. evaluated the use of DBS in 2015 [98] and established a reliable analytical method which is even used in a clincial trial investigating the costs and effects of pharmacogentic screening in nortriptyline therapy (ClincialTrial.gov Identifier NCT01778907).

3.2. Measuring patients' adherence

In 2003, adherence was declared by the World Health Organization as "the extent to which the persons' behavior (including medication-taking) corresponds with agreed recommendations from a healthcare provider" [99]. Diverse types of measuring patients' adherence are present today. None of those is defined as the gold standard, but in general they can be divided up into two areas (subjective or objective measurements). An example for an objective one is to analyze blood drug or metabolite concentrations [100], normally performed by venous blood sampling. Problems arise, when patients are forced to visit a physician for adherence testing or live in remote areas. Sampling at trough level is often challenging with venous blood withdrawal [101].

Tuberculosis is common in resource limited areas, making it challenging to transport plasma samples for adherence screening. DBS might be a cost-saving option. Allanson et al. established the use of DBS to avoid compliance-related problems under rifampicin therapy [102]. Another agent used to treat tuberculosis is moxifloxacin. When co-administered with rifampicin its plasma concentrations often decrease. Until now, the optimal dosage for treatment is not defined. Vu et al. established a method to quantify DBS samples of moxifloxacin to eradicate this effect and avoid inef-

fective blood levels [103]. In 2014 they further investigated DBS use to measure clarithromycin, rifampicin, and their metabolites. Special attention was paid to HTC, because tuberculosis patients tend to exhibit lower hematocrit values [104].

Miltefosine is a drug used to eradicate leishmaniasis, which is a disease present in remote states in Africa, Asia, and South America. A successful therapy depends on satisfactory drug exposure. To optimize therapy, Kip et al. introduced a method comparison of miltefosine in DBS. It is important to adjust calibration to low HTC values. At the beginning of the treatment, patients demonstrate lower hematocrit values of 25%, which gradually recovers over time [105].

Scherf-Clavel and Högger analyzed metformin, sitagliptin and creatinine in DBS to prevent non-adherence [106]. Both drugs are often co-administered and predominantly renal eliminated. Decreasing renal function (displayed by changing creatinine values) is often related to diabetes mellitus type 2. It worsens the drugs' clearance fundamentally and leads to non-adherence.

Enderle et al. investigated the use of DBS to prevent non-adherence or toxic side effects under ambrisentan, bosentan, sildenafil, tadalafil therapy against pulmonary hypertension. Notably, stability of these drugs was tested extensively under different conditions, e.g. DBS spots stored in direct sunlight. For the clinical application a conversion factor between DBS and plasma concentrations were determined, to be applicable for future concentration measurements [45].

3.3. Enabling children friendly blood sampling

Blood sampling for PK analysis in clinical trials with children is crucial and predominant for ethical considerations. The EMA recommends a maximum blood volume withdrawal of 1% of the child's total blood volume at each time point. Over a period of four weeks it should not exceed 3%. Deviations from the recommendation needs clear justification [107]. Even more, parents are restrictive to allow frequent blood collection [108]. Collecting blood samples with DBS may offer the opportunity to conduct more clinical trials with children.

Antiepileptic drugs are often characterized by a narrow therapeutic margin and monitoring of blood concentrations is recommendable. DBS sampling in pediatrics is an option to reduce the burden of recurrent blood withdrawal. La Marca et al. proved DBS to be useful for topiramate measurements [109]. The clinical trial was conducted in 3 days- to 73 year-old patients. Also the use of DBS for phenobarbital measurements in 2 days- to 81 year-old patients was investigated [110]. Measured DBS concentrations were multiplied with a factor (Table 1) to compare with plasma concentrations. For these calculations, hematocrit values were assumed to be 55% (mean in newborns) and 40% (mean in adults). Authors paid attention to extreme hematocrit values and announced that personal hematocrit should not be ignored. In 2011, further research on anticonvulsive medication in pediatrics was done with the recently introduced agent rufinamide [111]. At last, phenytoin was quantified with DBS in a pediatric population by Villanelli et al. [112].

Dickerson et al. investigated the influence of transportation from remote areas on DBS concentrations of tacrolimus and sirolimus for TDM in pediatrics. For this purpose, capillary blood was taken in clinic by a study nurse and children were send back home from hospital with their spotted DBS cards and instructed to send it back, when arriving. Further investigations were suggested to assess the influence of untrained sampling by patients themselves on analytics. In conclusion, home sampling was pronounced as a cost saving, reliable alternative [101].

HIV infected children are prone to varying drug concentrations, due to altering pharmacokinetics during early childhood development. Meesters et al. investigated the use of DBS for TDM measurements for lopinavir and ritonavir in pediatric patients. It was proven, that DBS is a useful tool and can be easily adopted also in remote areas [113].

Also altering drug concentrations are expected for children in chosen antibiotic therapies. Therefore, La Marca et al. established a DBS method to measure linezolid [114]. A formula (Table 1) converted DBS data into theoretical plasma concentrations. A comparable design was used by Della Bona et al. to establish pharmacokinetic studies with propranolol in children [115].

Acetaminophen is one of the most prescribed analgesics in pediatrics. Although, generally accepted as a safe drug its toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) causes liver failure. Taylor et al. investigated the use of DBS to determine acetaminophen blood concentrations to prevent toxic effects under pediatric treatment [108].

Methotrexate (MTX) is the most important first line therapy used against juvenile chronic inflammatory disorders. Side effects under therapy are common, because inter-patient variability to clinical response is often present. Its metabolites (MTX-polyglutamates) are suggested to be responsible for efficacy and side effects of MTX therapy. Monitoring MTX and its metabolites is beneficial for patient safety. Hawwa et al. presented a DBS method to evaluate its use for MTX and its polyglutamates (di- to pentaglutamate) monitoring [116].

3.4. Proofing DBS applicability in drug development

DBS application in pharmacokinetic studies experiences rising acceptance and offer several remarkable benefits [117]. Toxicological studies need fewer animals, blood volume, space and cooling capacity for sample storage [23]. Comprising all advantages, DBS supports the 3R rule for animal use in drug development (reduction, refinement and replacement) [8]. DBS sampling might save money, but advances to regulatory guidelines for DBS usage in drug approval is still quite slowly [118].

De Boer et al. made use of DBS for phenotyping and genotyping of CYP3A and used midazolam and its main metabolite 1-OH-midazolam as model substances for CYP3A4 activity [19]. To examine CYP2C9 activity, Déglon et al. established a DBS method to quantify blood concentrations of the substrate fluriprofen and its metabolite 4-hydroxyfluriprofen [119].

Xu et al. tested DBS applicability in currently running pharmacokinetic studies evaluating a new tyrosine kinase inhibitor (MK-1775) [120]. This paper illustrated the reliability of DBS during drug development and serves as a contribution for this ongoing discussion.

Another aspect for DBS application was considered by Page-Sharp et al. in 2015 [66]. To prevent the emergence of antimicrobial resistance, they investigated the use of DBS to quantify antibiotic blood concentrations. As an example ceftriaxone was used, because pharmacokinetic data for establishing optimal dosage regimes is missing.

4. Conclusion

The use of DBS in a clinical setting is an uprising issue. Since mass spectrometry becomes affordable also for clinical oriented laboratories, sensitive drug quantifications in complex matrices as required for DBS are no longer challenging [3]. All cited procedures were performed by mass spectrometry. Except from volatile analytes quantifications [67,95], liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) was used for drug quantification in DBS.

The first general quality control considerations on DBS were summarized by the EBF in 2011 [5] and streamlined the establishment of DBS methods more thoroughly onto specific validation procedures. All analytical and clinical guidelines and recommendations [5,24,25,36,62] for DBS method validations currently present, were perfectly considered by the following publications: Jager et al. for quantifying tamoxifen [40]; Page-Scharp et al. to analyze ceftriaxone [66]; Berm et al. to monitor critical tricyclic antidepressants [98], Kip et al. for measuring miltefosine in resource-limited areas [105], and Xu et al. in a clinical trial [120].

Concerning laboratory aspects, some papers described to cut out the whole spot [72,78,97,106,108]. Although no HTC testing is then needed, punching DBS is the most practical option to extract DBS.

On clinical aspect, it is indispensable to judge biological transferability. When considering DBS for TDM, adherence/persistence examination or clinical trials with children, paper spots are generated from capillary whole blood. As a future perspective, patients should be able to conduct DBS by themselves at home. This might de-stress health care systems and is a cost serving aspect [3]. Nevertheless, not every research group took care of this aspect when proving DBS for these purposes.

Concluding, up to date, no official guideline for the usage of DBS in clinic and/or (pre)clinical trials are legislated by any government. All reviewed paper described DBS' feasibly for clinical application. For clinical usage, it is prerequisite to evaluate the deviation between DBS measured concentrations to plasma measured concentrations. Most groups considered the use of a conversional factor to recalculate the difference. E.g. Enderle et al., further analyzed results with Bland-Altman to investigate, whether DBS measured concentrations are transferable to plasma concentrations [45].

Notably, a few papers investigated DBS implementation from patients' point of view. To ensure, that a new method is used in clinic, it is unavoidable to estimate patients' willingness to adapt the new sampling method. Van der Elst et al. and Kromdijk et al. considered this aspect deeply [71,91]. Despite all advantages, a new method can only be established if patient's acceptance is guaranteed. If DBS are supposed to be generated at home, scientists should regard to this aspect.

References

[1] A.J. Wilhelm, J.C. den Burger, E.L. Swart, Therapeutic drug monitoring by dried blood spots: progress to date and future directions, Clin. Pharmacokinet. 53 (2014) 961–973.
[2] M. Rowland, G.T. Emmons, Use of dried blood spots in drug development: pharmacokinetic considerations, AAPS 12 (2010) 290–293.
[3] M. Wagner, D. Tonoli, E. Varesio, G. Hopfgartner, The use of mass spectrometry to analyze dried blood spots, Mass Spectrom. Rev. 9999 (2014) 1–78.
[4] R. Guthrie, A. Susi, A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants, Pediatrics 32 (1963) 338–343.
[5] P. Timmerman, S. White, S. Globig, S. Lüdtke, L. Brunet, J. Smeraglia, EBF recommendation on the validation of bioanalytical methods for dried blood spots, Bioanalysis 3 (2011) 1567–1575.
[6] P.M. Edelbroek, J. van der Heijden, L.M.L. Stolk, Dried blood spot methods in therapeutic drug monitoring: methods, assays, and pitfalls, Ther. Drug Monit. 31 (2009) 327–336.
[7] R. Ter Heine, J.W. Mulder, E.C. van Gorp, J.F.P. Wagenaar, J.H. Beijnen, A.D. Huitema, Clinical evaluation of the determination of plasma concentrations of darunavir etravirine, raltegravir and ritonavir in dried blood spot samples, Bioanalysis 3 (2011) 1093–1097.
[8] European Union, Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes, 2010 (accessed 17.03.16) http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF.
[9] A. Sharma, S. Jaiswal, M. Shukla, J. Lal, Dried blood spots: concepts, present status, and future perspectives in bioanalysis, Drug Test. Anal. 6 (2014) 399–414.
[10] P. Denniff, N. Spooner, Volumetric absorptive microsampling: a dried sample collection technique for quantitative bioanalysis, Anal. Chem. 86 (2014) 8489–8495.
[11] A. Amara, L. Else, J. Tija, A. Olagunju, R.L. Puls, S. Khoo, D. Back, A. Validated, Method for quantification of efavirenz in dried blood spots using high-performance liquid chromatography–mass spectrometry, Ther. Drug Monit. 37 (2015) 220–228.
[12] R. Garcia Boy, Determination of morphine and 6-acetylmorphine in blood with use of dried blood spots, Ther. Drug Monit. 30 (2008) 733–739.
[13] W. Li, F.L. Tse, Dried blood spot sampling in combination with LC–MS/MS for quantitative analysis of small molecules, Biomed. Chromatogr. 24 (2010) 49–65.
[14] E. Saussereau, C. Lacroix, J.M. Gaulier, J.P. Goulle, On-line liquid chromatography/tandem mass spectrometry simultaneous determination of opiates, cocainics and amphetamines in dried blood spots, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 15 (2012) 1–7.
[15] P.A. Demirev, Dried blood spots: analysis and applications, Anal. Chem. 85 (2013) 779–789.
[16] S. Batterman, S. Chernyak, Performance and storage integrity of dried blood spots for PCB, BFR and pesticide measurements, Sci. Total Environ. 494–495 (2014) 252–260.
[17] A. Calcagno, I. Motta, M.G. Milia, R. Rostagno, M. Simiele, V. Libanore, S. Fontana, A. D'Avolio, V. Ghisetti, G. Di Perri, S. Bonora, Dried plasma/blood spots for monitoring antiretroviral treatment efficacy and pharmacokinetics: a cross-sectional study in rural Burundi, Br. J. Clin. Pharmacol. 79 (2015) 801–808.
[18] N. Gruner, O. Stambouli, R.S. Ross, Dried blood spots—preparing and processing for use in immunoassays and in molecular techniques, J. Vis. Exp. 97 (2015) 52619.
[19] T. de Boer, J. Wieling, E. Meulman, M. Reuvers, G. Renkema, I. den Daas, T. van Iersel, J. Wemer, L. Chen, Application of dried blood spot sampling combined with LC–MS/MS for genotyping and phenotyping of CYP450 enzymes in healthy volunteers, Biomed. Chromatogr. 25 (2011) 1112–1123.
[20] R.L. Hamers, P.W. Smit, W. Stevens, R. Schuurman, T.F. Rinke de Wit, Dried fluid spots for HIV type-1 viral load and resistance genotyping: a systematic review, Antivir. Ther. 14 (2009) 619–629.
[21] T. Roberts, H. Bygrave, E. Fajardo, N. Ford, Challenges and opportunities for the implementation of virological testing in resource-limited settings, J. Int. AIDS Soc. 15 (2012) 17324.
[22] R. Audu, C. Onwuamah, O. Salu, A. Okwuraiwe, C.Y. Ou, O. Bolu, K.B. Bond, K. Diallo, L. Lu, T. Jelpe, M. Okoye, E. Ngige, J. Vertefeuille, Development and implementation challenges of a quality assured HIV infant diagnosis program in Nigeria using dried blood spots and DNA polymerase chain reaction, AIDS Res. Hum. Retroviruses 31 (2015) 433–438.
[23] G. Emmons, M. Rowland, Pharmacokinetic considerations as to when to use dried blood spot sampling, Bioanalysis 2 (2010) 1791–1796.
[24] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), Guidance for Industry: Bioanalytical Method Validation, 2001 (accessed 17.03.16) http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf.
[25] European Medicines Agency, Guideline on Bioanalytical Method Validation, 2011 (accessed 17.03.16) http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf.
[26] W.D. van Dongen, L.C. Renfurm, Moving forward together: we are making progress, Bioanalysis 6 (2014) 1159–1165.
[27] E. Wickremsinhe, N.H. Huang, B.G. Abdul, K. Knotts, K.J. Ruterbories, J.R. Manro, Preclinical bridging studies: understanding dried blood spot and plasma exposure profiles, Bioanalysis 5 (2013) 159–170.
[28] C. Evans, M. Arnold, P. Bryan, J. Duggan, C.A. James, W. Li, S. Lowes, L. Matassa, T. Olah, P. Timmerman, X. Wang, E. Wickremsinhe, J. Williams, E. Woolf, P. Zane, Implementing dried blood spot sampling for clinical pharmacokinetic determinations: considerations from the IQ Consortium Microsampling Working Group, AAPS J. 17 (2015) 292–300.
[29] N.G. Jager, H. Rosing, J.H. Schellens, J.H. Beijnen, Procedures and practices for the validation of bioanalytical methods using dried blood spots: a review, Bioanalysis 6 (2014) 2481–2514.
[30] C.L. Bowen, W. Dopson, D.C. Kemp, M. Lewis, R. Lad, C. Overvold, Investigations into the environmental conditions experienced during ambient sample transport: impact to dried blood spot sample shipments, Bioanalysis 3 (2011) 1625–1633.
[31] A. Schwartz, A. Baidjoe, P.J. Rosenthal, G. Dorsey, T. Bousema, B. Greenhouse, The effect of storage and extraction methods on amplification of plasmodium falciparum DNA from dried blood spots, Am. J. Trop. Med. Hyg. 92 (2015) 922–925.
[32] N.G. Jager, H. Rosing, J.H. Schellens, J.H. Beijnen, Determination of tamoxifen and endoxifen in dried blood spots using LC–MS/MS and the effect of coated DBS cards on recovery and matrix effects, Bioanalysis 6 (2014) 2999–3009.
[33] B.L. van Baar, T. Verhaeghe, O. Heudi, M. Rohde, S. Wood, J. Wieling, R. de Vries, S. White, Z. Cobb, P. Timmerman, IS addition in bioanalysis of DBS: results from the EBF DBS-microsampling consortium, Bioanalysis 5 (2013) 2137–2145.
[34] P. Abu-Rabie, P. Denniff, N. Spooner, J. Brynjolffssen, P. Galluzzo, G. Sanders, Method of applying internal standard to dried matrix spot samples for use in quantitative bioanalysis, Anal. Chem. 83 (2011) 8779–8786.
[35] P. Abu-Rabie, P. Denniff, N. Spooner, B.Z. Chowdhry, F.S. Pullen, Investigation of different approaches to incorporating internal standard in DBS quantitative bioanalytical workflows and their effect on nullifying hematocrit-based assay bias, Anal. Chem. 87 (2015) 4996–5003.

[36] P. Timmerman, S. White, Z. Cobb, R. de Vries, E. Thomas, B. van Baar, Update of the EBF recommendation for the use of DBS in regulated bioanalysis integrating the conclusions from the EBF DBS-microsampling consortium, Bioanalysis 5 (2013) 2129–2136.
[37] R. de Vries, M. Barfield, N. van de Merbel, C. Siethoff, J. Ortiz, E. Verheij, B. van Baar, Z. Cobb, S. White, P. Timmerman, The effect of hematocrit on bioanalysis of DBS: results from the EBF DBS-microsampling consortium, Bioanalysis 5 (2013) 2147–2160.
[38] R.A. Koster, R. Botma, B. Greijdanus, D.R. Uges, J.G. Kosterink, D.J. Touw, J.W. Alffenaar, The performance of five different dreid blood spot cards for the analysis of six immunosuppressants, Bioanalysis 7 (2015) 1225–1235.
[39] Z. Cobb, R. de Vries, N. Spooner, S. Williams, L. Staelens, M. Doig, R. Broadhurst, M. Barfield, N. van de Merbel, B. Schmid, C. Siethoff, J. Ortiz, E. Verheij, B. van Baar, S. White, P. Timmerman, In-depth study of homogeneity in DBS using two different techniques: results from the EBF DBS-microsampling consortium, Bioanalysis 5 (2013) 2161–2169.
[40] N.G. Jager, H. Rosing, J.H. Schellens, J.H. Beijnen, S.C. Linn, Use of dried blood spots for the determination of serum concentrations of tamoxifen and endoxifen, Breast Cancer Res. Treat. 146 (2014) 137–144.
[41] D. de Wit, J. den Hartigh, H. Gelderblom, Y. Qian, M. den Hollander, H. Verheul, H.J. Guchelaar, N.P. van Erp, Dried blood spot analysis for therapeutic drug monitoring of pazopanib, J. Clin. Pharmacol. 55 (2015) 1344–1350.
[42] Fan, J.A. Lee, Managing the effect of hematocrit on DBS analysis in a regulated environment, Bioanalysis 4 (2012) 345–347.
[43] S. Capiau, V.V. Stove, W.E. Lambert, C.P. Stove, Prediction of the hematocrit of dried blood spots via potassium measurement on a routine clinical chemistry analyzer, Anal. Chem. 85 (2013) 404–410.
[44] R.A. Koster, J.W. Alffenaar, R. Botma, B. Geijdanus, D.J. Touw, D.R. Uges, J.G. Kosterink, What is the right blood hematocrit preparation procedure for standards and quality controls for dried blood spot analysis? Bioanalysis 7 (2015) 345–351.
[45] Y. Enderle, A.D. Meid, J. Friedrich, E. Grünig, H. Wilkens, W.E. Haefeli, J. Burhenne, Dried blood spot technique for the monitoring of ambrisentan, bosentan, sildenafil, and tadalafil in patients with pulmonary arterial hypertension, Anal. Chem. 15 (2015) 12112–12120.
[46] W. Li, J. Doherty, S. Favara, C. Breen, J. Flarakos, F.L. Tse, Evaluation of plasma microsampling for dried plasma spots (DPS) in quantitative LC–MS/MS bioanalysis using ritonavir as a model compound, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 991 (2015) 46–52.
[47] S.L. Parker, J. Lipman, G. Dimopoulos, J.A. Roberts, S.C. Wallis, A validated method for the quantification of fosfomycin on dried plasma spots by HPLC–MS/MS: application to a pilot pharmacokinetic study in humans, J. Pharm. Biomed. Anal. 115 (2015) 509–514.
[48] J.H. Kim, T. Woenker, J. Adamec, F.E. Regnier, Simple, miniaturized blood plasma extraction method, Anal. Chem. 85 (2013) 11501–11508.
[49] F. Li, J. Zulkoski, D. Fast, S. Michael, Perforated dried blood spots: a novel format for accurate microsampling, Bioanalysis 3 (2011) 2321–2333.
[50] N. Youhnovski, A. Bergeron, M. Furtado, F. Garofolo, Pre-cut dried blood spot (PCDBS): an alternative to dried blood spot (DBS) technique to overcome hematocrit impact, Rapid Commun. Mass Spectrom. 25 (2011) 2951–2958.
[51] R.J. Meesters, J. Zhan, N.A. van Huizen, G.P. Hooff, R.A. Gruters, T.M. Luider, Dried matrix on paper disks: the next generation DBS microsampling technique for managing the hematocrit effect in DBS analysis, Bioanalysis 4 (2012) 2027–2035.
[52] A.C. Rosypal, L.D. Pick, J.O. Hernandez, D.S. Lindsay, Evaluation of a novel dried blood spot collection device (HemaSpot) to test blood samples collected from dogs for antibodies to Leishmania infantum, Vet. Parasitol. 205 (2014) 338–342.
[53] K. Brooks, A. DeLong, M. Balamane, L. Schreier, M. Orido, M. Chepkenja, E. Kemboi, M. D'Antuono, P.A. Chan, W. Emonyi, L. Diero, M. Coetzer, R. Kantor, HemaSpot, a novel blood storage device for HIV-1 drug resistance testing, J. Clin. Microbiol. 54 (2016) 223–225.
[54] Spot on Sciences, 2016 (accessed 17.03.16) http://www.spotonsciences.com/.
[55] Spot on Sciences, Reducing the Hematocrit Effect for Dried Blood Spot Anaylsis, 2013 (accessed 17.03.16) http://www.spotonsciences.com/wp-content/uploads/AACC-Hct-poster-2013.pdf.
[56] S.D. Polley, D. Bell, J. Oliver, F. Tully, M.D. Perkins, P.L. Chiodini, I.J. Gonzalez, The design and evaluation of a shaped filter collection device to sample and store defined volume dried blood spots from finger pricks, Malar. J. 14 (2015) 45.
[57] Neoteryx, LLC. http://www.neoteryx.com/, 2015, (accessed 17.03.16).
[58] Z. Miao, J.G. Farnham, G. Hanson, T. Podoll, M.J. Reid, Bioanalysis of emixustat (ACU-4429) in whole blood collected with volumetric absorptive microsampling by LC–MS/MS, Bioanalyis 7 (2015) 2071–2083.
[59] L.A. Leuthold, O. Heudi, J. Deglon, M. Raccuglia, M. Augsburger, F. Picard, O. Kretz, A. Thomas, Anal. Chem. 87 (2015) 2068–2071.
[60] G. Lenk, S. Sandkvist, A. Pohanka, G. Stemme, O. Beck, N. Roxhed, New microfluidic-based sampling procedure for overcoming the hematocrit problem associated with dried blood spot analysis, Bioanalysis 7 (2015) 2085–2094.
[61] Trajan Scientific and Medical., 2016 (accessed 17.03.16) http://www.trajanscimed.com/pages/hemapen.
[62] Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, Second ed., Clinical and Laboratory Standards Institute, 2002.
[63] P.J. Cornbleet, N. Gochman, Incorrect least-squares regression coefficients in method comparison analysis, Clin. Chem. 25 (1979) 432–438.
[64] K. Linnet, Evaluation of regression procedures for methods comparison studies, Clin. Chem. 39 (1993) 424–432.
[65] H. Passing, W. Bablok, A new biometrical procedure for testing the equality of measurements from two different analytical methods, J. Clin. Chem. Clin. Biochem. 21 (1983) 709–720.
[66] M. Page-Sharp, T. Nunn, S. Salman, B.R. Moore, K.T. Batty, T.M. Davis, L. Manning, Validation and application of a dried blood spot ceftriaxone assay, Antimicrob. Agents Chemother. 60 (2015) 14–23.
[67] S.T. Kong, S.H. Lim, W.B. Lee, P.K. Kumar, H.Y. Wang, Y.L. Ng, P.S. Wong, P.C. Ho, Clinical validation and implications of dried blood spot sampling of carbamazepine, valproic acid and phenytoin in patients with epilepsy, PLoS One 9 (2014) e108190.
[68] D.G. Watson, E.J. Oliveira, A.C. Boyter, K.D. Dagg, A rapid and sensitive method for the determination of the amount of theophylline in blood spots, J. Pharm. Pharmacol. 53 (2001) 413–416.
[69] T. Koal, H. Burhenne, R. Romling, M. Svoboda, K. Resch, V. Kaever, Quantification of antiretroviral drugs in dried blood spot samples by means of liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 19 (2005) 2995–3001.
[70] W. Kromdijk, J.W. Mulder, H. Rosing, P.M. Smit, J.H. Beijnen, A.D. Huitema, Use of dried blood spots for the determination of plasma concentrations of nevirapine and efavirenz, J. Antimicrob. Chemother. 67 (2012) 1211–1216.
[71] W. Kromdijk, J.W. Mulder, P.M. Smit, R. Ter Heine, J.H. Beijnen, A.D. Huitema, Therapeutic drug monitoring of antiretroviral drugs at home using dried blood spots: a proof-of-concept study, Antivir. Ther. 18 (2013) 821–825.
[72] A. Olagunju, A. Amara, C. Waitt, L. Else, S.D. Penchala, O. Bolaji, J. Soyinka, M. Siccardi, D. Back, A. Owen, S. Khoo, Validation and clinical application of a method to quantify nevirapine in dried blood spots and dried breast-milk spots, J. Antimicrob. Chemother. 70 (2015) 2816–2822.
[73] J.R. Castillo-Mancilla, J.H. Zheng, J.E. Rower, A. Meditz, E.M. Gardner, J. Predhomme, C. Fernandez, J. Langness, J.J. Kiser, L.R. Bushman, P.L. Anderson, Tenofovir, emtricitabine, and tenofovir diphosphate in dried blood spots for determining recent and cumulative drug exposure, AIDS Res. Hum. Retroviruses 29 (2013) 384–390.
[74] J.H. Zheng, L.A. Guida, C. Rower, J. Castillo-Mancilla, A. Meditz, B. Klein, B.J. Kerr, J. Langness, L. Bushman, J. Kiser, P.L. Anderson, Quantitation of tenofovir and emtricitabine in dried blood spots (DBS) with LC–MS/MS, J. Pharm. Biomed. Anal. 88 (2014) 144–151.
[75] J.T. Hoffman, S.S. Rossi, R. Espina-Quinto, S. Letendre, E.V. Capparelli, Determination of efavirenz in human dried blood spots by reversed-phase high-performance liquid chromatography with UV-detection, Ther. Drug Monit. 35 (2013) 203–208.
[76] E. Kralj, J. Trontelj, T. Pajic, A. Kristl, Simultaneous measurement of imatinib, nilotinib and dasatinib in dried blood spot by ultra high performance liquid chromatography tandem mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 903 (2012) 150–156.
[77] B.S. Mohammed, G.A. Cameron, L. Cameron, G.H. Hawksworth, P.J. Helms, J.S. McLay, Can finger-prick sampling replace venous sampling to determine the pharmacokinetic profile of oral paracetamol? Br. J. Clin. Pharmacol. 70 (2010) 52–56.
[78] R. Rezonja Kukec, I. Grabnar, A. Mrhar, N. Cebron Lipovec, T. Cufer, T. Vovk, A simple dried blood spot method for clinical pharmacological analyses of etoposide in cancer patients using liquid chromatography and fluorescence detection, Clin. Chim. Acta 452 (2016) 99–105.
[79] M.V. Antunes, R. Linden, T.V. Santos, P. Wallemacq, V. Haufroid, J.F. Classen, H. Andreolla, N. Costa, T.O. Fontanive, D.D. Rosa, Endoxifen levels and its association with CYP2D6 genotype and phenotype: evaluation of a southern Brazilian population under tamoxifen pharmacotherapy, Ther. Drug Monit. 34 (2012) 422–431.
[80] M.V. Antunes, S. Raymundo, V. de Oliveira, D.E. Staudt, G. Gossling, G.P. Peteffi, J.V. Biazus, J.A. Cavalheiro, M. Tre-Hardy, A. Capron, V. Haufroid, P. Wallemacq, G. Schwartsmann, R. Linden, Ultra-high performance liquid chromatography tandem mass spectrometric method for the determination of tamoxifen, *N*-desmethyltamoxifen, 4-hydroxytamoxifen and endoxifen in dried blood spots—development, validation and clinical application during breast cancer adjuvant therapy, Talanta 132 (2015) 775–784.
[81] E. Hinchliffe, J.E. Adaway, B.G. Keevil, Simultaneous measurement of cyclosporin A and tacrolimus from dried blood spots by ultra high performance liquid chromatography tandem mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 883–884 (2012) 102–107.
[82] B.G. Keevil, J. Fildes, A. Baynes, N. Yonan, Liquid chromatography–mass spectrometry measurement of tacrolimus in finger-prick samples compared with venous whole blood samples, Ann. Clin. Biochem. 46 (2009) 144–145.
[83] K. Sadilkova, B. Busby, J.A. Dickerson, J.C. Rutledge, R.M. Jack, Clinical validation and implementation of a multiplexed immunosuppressant assay in dried blood spots by LC–MS/MS, Clin. Chim. Acta 421 (2013) 152–156.
[84] R.A. Koster, J.W. Alffenaar, B. Greijdanus, D.R. Uges, Fast LC–MS/MS analysis of tacrolimus sirolimus, everolimus and cyclosporin A in dried blood spots and the influence of the hematocrit and immunosuppressant concentration on recovery, Talanta 115 (2013) 47–54.
[85] D. Koop, L.A. Bleyle, M. Munar, G. Cherala, A. Al-Uzri, Analysis of tacrolimus and creatinine from a single dried blood spot using liquid chromatography tandem mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 926 (2013) 54–61.

[86] I.R. Dubbelboer, A. Pohanka, R. Said, S. Rosenborg, O. Beck, Quantification of tacrolimus and three demethylated metabolites in human whole blood using LC-ESI-MS/MS, Ther. Drug Monit. 34 (2012) 134–142.
[87] Q. Li, D. Cao, Y. Huang, H. Xu, C. Yu, Z. Li, Development and validation of a sensitive LC–MS/MS method for determination of tacrolimus on dried blood spots, Biomed. Chromatogr. 27 (2013) 327–334.
[88] A.J. Wilhelm, J.C. den Burger, R.M. Vos, A. Chahbouni, A. Sinjewel, Analysis of cyclosporin A in dried blood spots using liquid chromatography tandem mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877 (2009) 1595–1598.
[89] A.J. Wilhelm, Clinical validation of dried blood spot sampling in therapeutic drug monitoring of ciclosporin a in allogeneic stem cell transplant recipients: direct comparison between capillary and venous sampling, Ther. Drug Monit. 35 (2013) 92–95.
[90] J. Arpini, M.V. Antunes, L.S. Pacheco, D. Gnatta, M.F. Rodrigues, E. Keitel, R. Linden, Clinical evaluation of a dried blood spot method for determination of mycophenolic acid in renal transplant patients, Clin. Biochem. 46 (2013) 1905–1908.
[91] K.C. van der Elst, L.F. Span, K. van Hateren, K.M. Vermeulen, T.S. van der Werf, B. Greijdanus, J.G. Kosterink, D.R. Uges, J.W. Alffenaar, Dried blood spot analysis suitable for therapeutic drug monitoring of voriconazole, fluconazole, and posaconazole, Antimicrob. Agents Chemother. 57 (2013) 4999–5004.
[92] D. Felce, J. Perry, Quality of life: its definition and measurement, Res. Dev. Disabil. 16 (1995) 51–74.
[93] S.R. Cohen, B.M. Mount, J.J.N. Tomas, L.F. Mount, Existential well-being is an important determinant of quality of life, Am. Cancer Soc. 77 (1996) 576–586.
[94] N. Kostic, Y. Dotsikas, N. Jovic, G. Stevanovic, A. Malenovic, M. Medenica, Quantitation of pregabalin in dried blood spots and dried plasma spots by validated LC–MS/MS methods, J. Pharm. Biomed. Anal. 109 (2015) 79–84.
[95] L. Rhoden, M.V. Antunes, P. Hidalgo, C. Alvares da Silva, R. Linden, Simple procedure for determination of valproic acid in dried blood spots by gas chromatography–mass spectrometry, J. Pharm. Biomed. Anal. 96 (2014) 207–212.
[96] D. Lejeune, I. Souletie, S. Houze, T. Le bricon, J. Le bras, B. Gourmel, P. Houze, Simultaneous determination of monodesethylchloroquine chloroquine, cycloguanil and proguanil on dried blood spots by reverse-phase liquid chromatography, J. Pharm. Biomed. Anal. 43 (2007) 1106–1115.
[97] M.A. Saracino, G. Lazzara, B. Prugnoli, M.A. Raggi, Rapid assays of clozapine and its metabolites in dried blood spots by liquid chromatography and microextraction by packed sorbent procedure, J. Chromatogr. A 1218 (2011) 2153–2159.
[98] E.J. Berm, J. Paardekooper, E. Brummel-Mulder, E. Hak, B. Wilffert, J.G. Maring, A simple dried blood spot method for therapeutic drug monitoring of the tricyclic antidepressants amitriptyline, nortriptyline, imipramine, clomipramine, and their active metabolites using LC–MS/MS, Talanta 134 (2015) 165–172.
[99] World Health Organization, E. Sabaté, Adherence to Long-term Therapies: Evidence for Action, 2003 (accessed 17.03.16) http://apps.who.int/iris/bitstream/10665/42682/1/9241545992.pdf.
[100] W.Y. Lam, P. Fresco, Medication adherence measures: an overview, Biomed Res. Int. 2015 (2015) 217047.
[101] J.A. Dickerson, M. Sinkey, K. Jacot, J. Stack, K. Sadilkova, Y.M. Law, R.M. Jack, Tacrolimus and sirolimus in capillary dried blood spots allows for remote monitoring, Pediatr. Transplant. 19 (2015) 101–106.
[102] A.L. Allanson, M.M. Cotton, J.N. Tettey, A.C. Boyter, Determination of rifampicin in human plasma and blood spots by high performance liquid chromatography with UV detection: a potential method for therapeutic drug monitoring, J. Pharm. Biomed. Anal. 44 (2007) 963–969.
[103] D.H. Vu, R.A. Koster, J.W. Alffenaar, J.R. Brouwers, D.R. Uges, Determination of moxifloxacin in dried blood spots using LC–MS/MS and the impact of the hematocrit and blood volume, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 879 (2011) 1063–1070.
[104] D.H. Vu, R.A. Koster, M.S. Bolhuis, B. Greijdanus, R.V. Altena, D.H. Nguyen, J.R. Brouwers, D.R. Uges, J.W. Alffenaar, Simultaneous determination of rifampicin, clarithromycin and their metabolites in dried blood spots using LC–MS/MS, Talanta 121 (2014) 9–17.
[105] A.E. Kip, H. Rosing, M.J. Hillebrand, S. Blesson, B. Mengesha, E. Diro, A. Hailu, J.H. Schellens, J.H. Beijnen, T.P. Dorlo, Validation and clinical evaluation of a novel method to measure miltefosine in leishmaniasis patients using dried blood spot sample collection, Antimicrob. Agents Chemother. 60 (2016) 2081–2089.
[106] M. Scherf-Clavel, P. Högger, Analysis of metformin, sitagliptin and creatinine in human dried blood spots, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 997 (2015) 218–228.
[107] European Medicines Agency, Ethical Considerations for Clincial Trials on Medicinal Products Conducted with the Paediatric Population, 2008 (accessed 17.03.16) ftp://ftp.cordis.europa.eu/pub/fp7/docs/ethical-considerations-paediatrics_en.pdf.
[108] R.R. Taylor, K.L. Hoffman, B. Schniedewind, C. Clavijo, J.L. Galinkin, U. Christians, Comparison of the quantification of acetaminophen in plasma, cerebrospinal fluid and dried blood spots using high-performance liquid chromatography-tandem mass spectrometry, J. Pharm. Biomed. Anal. 83 (2013) 1–9.
[109] G. la Marca, S. Malvagia, L. Filippi, P. Fiorini, M. Innocenti, F. Luceri, G. Pieraccini, G. Moneti, S. Francese, F.R. Dani, R. Guerrini, Rapid assay of topiramate in dried blood spots by a new liquid chromatography-tandem mass spectrometric method, J. Pharm. Biomed. Anal. 48 (2008) 1392–1396.
[110] G. la Marca, S. Malvagia, L. Filippi, F. Luceri, G. Moneti, R. Guerrini, A new rapid micromethod for the assay of phenobarbital from dried blood spots by LC-tandem mass spectrometry, Epilepsia 50 (2009) 2658–2662.
[111] G. la Marca, S. Malvagia, L. Filippi, M. Innocenti, A. Rosati, M. Falchi, S. Pellacani, G. Moneti, R. Guerrini, Rapid assay of rufinamide in dried blood spots by a new liquid chromatography-tandem mass spectrometric method, J. Pharm. Biomed. Anal. 54 (2011) 192–197.
[112] F. Villanelli, E. Giocaliere, S. Malvagia, A. Rosati, G. Forni, S. Funghini, E. Shokry, D. Ombrone, M.L. Della Bona, R. Guerrini, G. la Marca, Dried blood spot assay for the quantification of phenytoin using liquid chromatography–mass spectrometry, Clin. Chim. Acta 440 (2015) 31–35.
[113] R.J. Meesters, J.J. van Kampen, M.L. Reedijk, R.D. Scheuer, L.J. Dekker, D.M. Burger, N.G. Hartwig, A.D. Osterhaus, T.M. Luider, R.A. Gruters, Ultrafast and high-throughput mass spectrometric assay for therapeutic drug monitoring of antiretroviral drugs in pediatric HIV-1 infection applying dried blood spots, Anal. Bioanal. Chem. 398 (2010) 319–328.
[114] G. la Marca, F. Villanelli, S. Malvagia, D. Ombrone, S. Funghini, M. De Gaudio, S. Fallani, M.I. Cassetta, A. Novelli, E. Chiappini, M. de Martino, L. Galli, Rapid and sensitive LC–MS/MS method for the analysis of antibiotic linezolid on dried blood spot, J. Pharm. Biomed. Anal. 67–68 (2012) 86–91.
[115] M.L. Della Bona, S. Malvagia, F. Villanelli, E. Giocaliere, D. Ombrone, S. Funghini, L. Filippi, G. Cavallaro, P. Bagnoli, R. Guerrini, G. la Marca, A rapid liquid chromatography tandem mass spectrometry-based method for measuring propranolol on dried blood spots, J. Pharm. Biomed. Anal. 78–79 (2013) 34–38.
[116] A.F. Hawwa, A. Albawab, M. Rooney, L.R. Wedderburn, M.W. Beresford, J.C. McElnay, A novel dried blood spot-LCMS method for the quantification of methotrexate polyglutamates as a potential marker for methotrexate use in children, PLoS One 9 (2014) e89908.
[117] N. Spooner, R. Lad, M. Barfield, Dried blood spot as a sample collection technique for the determination of pharmacokinetics in clinical studies: considerations for the validation of a quantitative bioanalytical method, Anal. Chem. 81 (2009) 1557–1563.
[118] P.A. Kothare, K.P. Bateman, M. Dockendorf, J. Stone, Y. Xu, E.J. Woolf, L.A. Shipley, An integrated strategy for implementation of dried blood spots in clinical development programs, AAPS 18 (2016) 519–527.
[119] J. Deglon, A. Thomas, Y. Daali, E. Lauer, C. Samer, J. Desmeules, P. Dayer, P. Mangin, C. Staub, Automated system for on-line desorption of dried blood spots applied to LC–MS/MS pharmacokinetic study of fluriprofen and its metabolite, J. Pharm. Biomed. Anal. 54 (2011) 359–367.
[120] Y. Xu, W. Fang, W. Zeng, S. Leijen, E.J. Woolf, Evaluation of dried blood spot (DBS) technology versus plasma analysis for the determination of MK-1775 by HILIC-MS/MS in support of clinical studies, Anal. Bioanal. Chem. 404 (2012) 3037–3048.
[121] R. ter Heine, H. Rosing, E.C. van Gorp, J.W. Mulder, W.A. van der Steeg, J.H. Beijnen, A.D. Huitema, Quantification of protease inhibitors and non-nucleoside reverse transcriptase inhibitors in dried blood spots by liquid chromatography-triple quadrupole mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 867 (2008) 205–212.