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MINIREVIEW

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Development and clinical applications of the dried blood spot method for therapeutic drug monitoring of anti-epileptic drugs

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Abstract

Anti-epileptic drugs (AEDs) have various pharmacokinetic profiles, inter-individual variabilities, high possibilities of drug-drug interactions and narrow therapeutic indices. To provide optimal treatment for patients, therapeutic drug monitoring (TDM) is necessary. However, TDM requires sufficient quantities of blood samples to measure drug concentrations. Therefore, TDM could be a burden, particularly in paediatric cases. A good alternative that overcomes these disadvantages is the dried blood spot (DBS) method, which is simple, convenient to use and less invasive, requiring a lower quantity of blood than traditional blood sampling methods. However, the DBS method is affected by haematocrit (Hct) levels to varying extents depending on the drug properties. In addition, different papers with varying characteristics are available for use when applying the DBS method. Therefore, it has not yet been applied to TDM in clinical practice. To achieve this, several steps are required, including method development, method validation and clinical validation. Currently, the development status of the DBS method is different for each AED and unclear. Therefore, we assessed the development status of the following 19 AEDs in 26 studies: lamotrigine, valproic acid, levetiracetam, phenytoin, topiramate, carbamazepine, carbamazepine epoxide, gabapentin, phenobarbital, pregabalin, clobazam, clonazepam, ethosuximide, felbamate, monohydroxycarbamazepine, nitrazepam, rufinamide, vigabatrin and zonisamide. Among them, carbamazepine, lamotrigine, topiramate and valproic acid have been developed such that they are nearly available for TDM. In addition, Whatman 903 Protein Saver Cards and concentration analysis by liquid chromatography with triple quadrupole mass spectrometer were used most often.

KEYWORDS

AED, anti-epileptic drugs, DBS, dried blood spot, TDM, therapeutic drug monitoring

1 | INTRODUCTION AND BACKGROUND

Therapeutic drug monitoring (TDM) is a method involving dose adjustment according to pharmacokinetics (PK) or pharmacodynamics (PD) to optimize patient outcomes, thereby individualizing treatment[1]. Anti-epileptic drugs (AEDs) have complex PK and PD, high inter-individual variabilities and, in the case of four particular AEDs, carbamazepine, phenobarbital, phenytoin and valproic acid, narrow therapeutic indices.[2,3] In addition, several AEDs are associated with cytochrome P450 enzyme metabolism, which can induce drug-drug interactions.[4] Therefore, TDM is usually conducted in patients with epilepsy to prevent seizures and reduce adverse events.[5] However, the traditional blood sampling method used for TDM is invasive

and painful and could fail for various reasons; thus, it is a potential burden, particularly in paediatrics.[6,7] In addition, venepuncture must be conducted by certified professionals, who process the blood samples to extract plasma and store them at freezing temperatures.[8] In contrast with traditional blood sampling, the dried blood spot (DBS) method has numerous advantages. For example, it enables blood collection by finger prick, which is less invasive than traditional blood sampling.[9] In addition, it requires only a small amount of blood, and the samples can be easily handled and stored.[8,10] Because of these advantages, the DBS method could be an alternative to traditional blood sampling.[11] PK studies have demonstrated that collecting a large volume of blood is not possible for paediatric patients[12]; therefore, the DBS method could be beneficial for this population because it is less invasive, requires a small amount of blood[13] and causes less discomfort.[12] Despite the advantages of using the DBS method, its application to TDM has been limited to only a few drugs. Therefore, the development status of the DBS method for each AED and the possibility of its clinical application to TDM need to be assessed.

2 | SEARCH STRATEGY

A literature search was conducted in journals published from April 1984 to October 2018 using PubMed, EMBASE and Scopus, which was limited to human studies that were written in English. The search terms reflecting DBS or dried plasma spot (DPS) were as follows: "Dried Blood Spot Testing", "Dr* blood spot,*" "Dr* plasma spot,*" "DBS," and "DPS." Those reflecting TDM were as follows: "Drug Monitoring," "Therapeutic drug monitoring," or "TDM." From the results, studies that included AEDs were selected manually. The results were confirmed by two independent authors (MKL and RJY).

3 | SUITABLE DRUG CHARACTERISTICS FOR DBS SAMPLING

1. The primary factor identified that could affect the DBS method was the haematocrit (Hct) level. Therefore, drugs with low protein-binding properties are less likely to be affected by Hct.[14]
2. Another important factor was the stability of the DBS sample at room temperature. Suitable drying times vary according to the literature from 15 minutes to overnight (the recommended time was ≥2 hours).[7]
3. A correlation coefficient [r] of $r^2 > 0.99$ between the drug concentrations of a DBS (C_{dbs}) and plasma (C_p) sample was suitable.[15]

4 | SAMPLE PREPARATION OF DBS

4.1 | Choice of DBS paper

DBS papers differ in their characteristics, including their matrices, pore sizes and thicknesses.[16] In previous studies, several types of DBS papers have been used. However, currently, only the following papers are commercially available: Whatman® (903 Protein Saver Card, FTA® DMPK-A, -B and -C, 31 ET CHR and Grade 3 filter paper), Ahlstrom® (AutoCollect® and BioSample TFN®), PerkinElmer® 226, Agilent Bond Elut DMS® and HSA-FMDP® (Blue Card and Red Card). Whatman 903®, PerkinElmer® 226 and Agilent Bond Elut DMS® were approved by the US Food and Drug Administration (FDA) as class 2 devices. The Whatman® 903 Protein Saver Card is most commonly used in studies applying the DBS method. It was initially developed for neonatal screening and is composed of five 12.7-mm circles, each which can hold 75-80 μL of blood.[17] Ahlstrom® has developed two types of DBS cards, called AutoCollect® and BioSample TFN®.[18] AutoCollect® is designed for PK/toxicokinetic, drug metabolism and pre-clinical and clinical studies using liquid chromatography mass spectrometer (LC-MS) analysis. BioSample TFN® is designed for screening infectious diseases, such as human immunodeficiency virus (HIV) and hepatitis, pre-clinical and clinical studies and drug monitoring. FTA DMPK® cards are made of cellulose and can contain up to 50 μL of blood per circle. FTA DMPK-A® and FTA DMPK-B® cards are coated by chemicals that lyse cells and denature proteins[19]; in contrast, FTA DMPK-C® cards and PerkinElmer® 226 cards are not chemically coated and thus are more suitable for protein-based bioanalysis.[16,20] In a previous study, four types of DBS paper were tested at different Hct levels using five compounds,[14] each with different protein-binding properties. Results demonstrated that the concentration bias caused by Hct levels was more pronounced in drugs with high protein-binding characteristics, particularly in chemically untreated papers.

DBS cards have been compared in studies on HIV virus load; in contrast, only a few studies on AEDs have been conducted.[21-23] Ikeda et al[24] compared Whatman FTA DMPK-A® and Bond Elut DMS® using valproic acid and gabapentin. Both cards demonstrated acceptable precision and accuracy, with DPS ranges of 10-200 μg/mL for valproic acid and 0.5-10 μg/mL for gabapentin (calibration curve $r^2 > 0.99$). However, the AEDs were unstable at 30°C in both cards; therefore, these should be stored in a cooler space or freezer. Moreover, the reliability of results using the Bond Elut DMS® card depended on applying consistent spotting volumes and punching positions. Linder et al[25] compared four filter papers, including the Whatman® 903 filter paper, Ahlstrom 226® filter paper, Whatman

903® Protein Saver Card and Whatman® FTA DMPK-C® card, using valproic acid, carbamazepine and lamotrigine. The coefficients of variation of all four papers were <5.6%; however, detailed data were not reported.

4.2 | General sampling procedure

The general DBS sampling procedure is simple and as follows:

1. Firstly, a small amount of blood spot (10-50 μL) is placed onto the DBS paper.
2. The card is dried for a sufficient period of time (≥2 hours) to enhance the stability of the samples.
3. The sample area is punched (usually at the centre of the circle) and placed in a tube.
4. The drug is extracted with a solution (eg methanol, acetonitrile, and water) containing the internal standard.
5. The solution is then mixed with a vortex mixer or orbital shaker for 5-10 minutes, and the impurities are removed with a centrifuge.
6. The supernatant is then subjected to liquid chromatography with triple quadrupole mass spectrometer (LC-MS/MS) or any other appropriate method for further analysis.

One study comparing four types of DBS paper (FTA DMPK-A®, FTA DMPK-B® and FTA DMPK-C® and Ahlstrom 226®) using five compounds with different protein-binding properties[14] found differences in the concentrations and distances between the perimeter and centre of the DBS between each paper. This suggests that it is important for the punch to be positioned consistently to ensure precision and accuracy.

4.3 | Effects of Hct levels

Hct level is the most critical factor that can affect the DBS sampling method[26] and affects spotting, drying time, homogeneity and concentration bias.[14,26,27] Smaller sample areas have been reported with higher Hct levels as a result of blood viscosity, with a linear relationship demonstrated for an Hct level of 0.25-0.75.[27] The concentration bias was greater when the Hct level was higher, and this tendency increased for compounds that had high protein-binding properties.[14] Therefore, the specific Hct range for each AED, particularly those with high protein-binding properties, should be evaluated before using the DBS method. The following equation has been used widely to convert the DBS concentration (C_{dbs}) to the plasma concentration (C_p).

$$\text{Theoretical Cp} = \frac{\text{Cdbs}}{[1-\text{Hct}(1-K)]},$$

where K indicates the AED ratio of red blood cells to plasma,[28] and two concentrations of some AEDs could be switched without calculation. The effect of the Hct level on each AED is summarized in Table 1.

4.4 | Venous vs capillary blood

Venous and capillary blood can have different matrix compositions and have been compared in various studies.[29-31] Lacher et al[29] compared the venous and DBS samples obtained by finger prick in 401 participants. The haemoglobin A1c, C-reactive protein and glucose levels were comparable between the venous and capillary blood; however, total and high-density lipoprotein cholesterol levels demonstrated a low correlation. Patel et al[30] also compared haemoglobin levels in venous and capillary blood obtained by finger-prick samples in 50 participants and found a significantly higher haemoglobin level in capillary blood than in venous blood. A study of healthy participants (six males and six females) compared the deformability and aggregation of red blood cells in venous and capillary blood obtained by finger-prick sampling.[31] The Hct levels and red blood cell deformability and aggregation were not significantly different between the samples; however, it was difficult to convert the drug concentrations for both. Additional studies comparing venous and capillary blood should be conducted to address the divergent results of previous studies.

There have been few studies comparing venous and capillary blood in patients with epilepsy. Hahn et al[32] compared topiramate concentrations between DBS samples obtained by finger prick and that of venous blood. Findings were similar after the distribution phase; however, the topiramate concentration was 6.5% higher in finger-prick blood samples than in venous blood. Other studies comparing venous and capillary blood concentrations of carbamazepine, lamotrigine and valproic acid also reported significant differences,[25] with six quality controls indicating higher concentrations in capillary blood than in venous blood.

5 | ADVANTAGES AND DISADVANTAGES OF DBS

The greatest advantage of the DBS method is that it requires a very small sample for analysis; therefore, there is no need to collect a large volume of venous blood. It is very useful for individuals who cannot easily provide a blood sample by injection, such as those with weak blood vessels, pregnant women, children, the elderly and patients with severe diseases. In addition to high patient satisfaction, the DBS method creates little human-derived waste and is thus environmentally friendly.[33] Storage of DBS paper requires only an environment with low humidity and temperature, which makes it stable, convenient and economical.[7] In addition, DBS specimens can be collected anywhere, even if patients

TABLE 1 Overview of analytical methods for AEDs using DBS

Medicine	Internal standard	Analytical method	Analytical range	Calibrator or QC DBS sampling method	Clinical DBS sampling method	Punch sizes (mm)	Paper type
Carbamazepine[28]	5-Methylphenyl hydantoin	GC-MS	0.5-120 μg/mL	Spiked blood was spotted onto paper (30 μL)	Two drops of blood (~30 μL each) were spotted onto paper	6	Whatman 903 Protein Saver cards
Carbamazepine[39]	-	GC-MS	-	Spiked EDTA and two 30 μL drops of venous blood were spotted onto paper (10 μL)	Spiked EDTA and two 30 μL drops of venous blood were spotted onto paper (10 μL)	6	Whatman 903 Protein Saver cards
Carbamazepine[40]	Carbamazepine-d10	LC-MS/MS	2.5-80 μmol/L	Spiked blood was spotted onto paper (30 μL)	Blood was spotted onto paper (30 μL)	4.7	Whatman 903 filter paper and protein saver card
Carbamazepine[41]	Carbamazepine-d8	LC-MS/MS	0.25-40 μg/mL	Spiked EDTA blood was spotted onto paper (30 μL)	3-5 Drops of blood was spotted onto paper by finger prick	3	Whatman 903 Protein Saver cards
Carbamazepine[42]	Nevirapine	HPLC	2-15 μg/mL	Spiked EDTA blood was spotted onto paper (20 μL)	EDTA blood was spotted onto paper (20 μL)	-	Whatman cellulose filter paper
Carbamazepine[44]	Carbamazepine-d10	LC-MS/MS	1-40 mg/L	Spiked blood was spotted onto paper (20 μL)	Blood was spotted onto paper (20 μL)	3.2	Whatman 903 Protein Saver cards
Carbamazepine[25]	Carbamazepine-d8	LC-MS/MS	0.25-40 μg/mL	Spiked EDTA blood was spotted onto paper (30 μL)	-	3	Whatman 903 filter paper

Sample volume (μL)	DBS sample extraction method	Dry time (h)	Comparison between DBS and plasma	Clinical sample	Hct effect	Method validation
60	One disc was extracted with 500 μL of ACN and 1 mol/L sodium hydroxide (24:1, v/v) containing IS Vortex for 1 min Sonication for 5 min Centrifuge for 15 min at 6000 *g*	3	Direct conversion available $C_p = (0.89 * C_{dbs}) + 1.00$ μg/mL	165 PWE who were on CBZ, VPA or/and PHT recruited from October 2011 to August 2012 from a neurology specialist clinic of a tertiary referral hospital	No Hct effect	FDA
60	One disc was extracted with alkalinized organic solvent comprised of 20 μL of sodium hydroxide 1 mol/L in 480 μL of CAN Vortex for 1 min Sonicate 5 min Centrifuge at 6000 *g* for 15 min	3	C_{dbs} and C_p could be interchangeable	97 PWE who contributed 98 observations	-	FDA
30	One disc was extracted with 200 μL of MeOH/water (65:35, v/v) containing IS Microplate shaker at 450 rpm for 30 min	3	21% higher in DBS. ($r^2 = 0.9274$, $y = 1.661x + 1.2078$)	Leftover blood from routine samples from patients on CBZ, LTG, LEV and VPA was used to produce DBS samples from venous or capillary blood	Acceptable in Hct range of 30%-50%	EMA
-	One disc was extracted with 200 μL of MeOH/water (80:20, v/v) containing IS Orbital shaker at 570 rpm for 5 min Centrifuge at 2100 *g* for 5 min	3	$C_p = 0.84 * C_{dbs}$	46 neuropaediatric patients aged 2-18 y	Acceptable in Hct range of 30%-55%	EMA
20	One disc was extracted with 160 μL of ACN: MeOH: ultrapure water (40:40:20) containing IS Vortex for 1 min Centrifuge at 9447 *g* for 5 min	Overnight	Good correlation between C_s and C_{dbs} ($r = 0.932$) $C_s = C_{dbs} \times 0.83 + 1.09$	80 PWE (80 specimen)	-	FDA
20	One disc was extracted with 200 μL of MeOH-water-formic acid (80:20:0.1,v/v/v) containing IS Orbital shaker for 25 min at 37℃	0.5	Good correlation ($r^2 = 0.964$) $C_p = C_{dbs} * (100/100 - Hct)$	19 Paired plasma and DBS samples from 12 patients aged 2 mo to 18 y, treated with CBZ	Acceptable in Hct range of 30%-50%	FDA, ICH
30	One disc was extracted with 200 μL of MeOH/water (80:20, v/v) containing IS Orbital shaker at 570 rpm for 5 min Centrifuge at 2100 *g* for 5 min	3	-	Healthy volunteers	Acceptable in Hct range of 30%-60%	EMA

(Continues)

TABLE 1 (Continued)

Medicine	Internal standard	Analytical method	Analytical range	Calibrator or QC DBS sampling method	Clinical DBS sampling method	Punch sizes (mm)	Paper type
Carbamazepine[34]	Hexobarbital	HPLC	1-17.5 μg/mL	Spiked blood was spotted onto paper (30 μL)	-	6	Schleicher and Schuell 903 Guthrie cards
Carbamazepine epoxide[44]	Carbamazepine epoxide-d10	LC-MS/MS	0.25-20 mg/L	Spiked blood was spotted onto paper (20 μL)	Blood was spotted onto paper in 20 μL	3.2	Whatman 903 protein saver cards
Carbamazepine epoxide[34]	Hexobarbital	HPLC	0.5-10 μg/mL	Spiked blood was spotted onto paper (30 μL)	-	6	Schleicher and Schuell 903 Guthrie cards
Clobazam[53]	Nordiazepam-d5	LC-MS/MS	20-5000 ng/mL	Spiked blood was spotted onto paper (5 μL)	Blood was spotted onto paper (5 μL)	6	Whatman 903 Protein Saver cards
Clonazepam[53]	Nordiazepam-d5	LC-MS/MS	0.5-100 ng/mL	Spiked blood was spotted onto paper (5 μL)	Blood was spotted onto paper (5 μL)	6	Whatman 903 Protein Saver cards
Ethosuximide[46]	Linezolid	HPLC	9.6-192 μg/mL	Spiked plasma was spotted onto paper (150 μL)	Plasma was spotted onto paper (150 μL)	-	Dried sample spot devices (DSSD) from Laboratori Biomicron
Felbamate[46]	Linezolid	HPLC	9.6-192 μg/mL	Spiked plasma was spotted onto paper (150 μL)	Plasma was spotted onto paper (150 μL)	-	Dried sample spot devices (DSSD) from Laboratori Biomicron
Gabapentin[43]	Gabapentin-d10	GC-MS	1-30 μg/mL	Spiked venous blood was spotted onto paper (25 μL)	A drop of blood was spotted onto paper by finger prick	6	Whatman 903 filter paper
Gabapentin[24]	Gabapentin-d4	GC-MS	0.5-10 μg/mL	Spiked plasma was spotted onto paper (20 μL)	Plasma was spotted onto paper (20 μL)	8	Whatman FTA DMPK-A cards or Bond Elut DMS card

Sample volume (μL)	DBS sample extraction method	Dry time (h)	Comparison between DBS and plasma	Clinical sample	Hct effect	Method validation
50	One disc was extracted with 980 μL of MeOH:ACN (3:1, v/v) containing IS Sonication for 15 min	Overnight	-	-	Minimal effect of Hct range of 30%-55%	ICH
20	One disc was extracted with 200 μL of MeOH-water-formic acid (80:20:0.1,v/v/v) containing IS Orbital shaker for 25 min at 37℃	0.5	Good correlation ($r^2 = 0.986$) $C_p = C_{dbs}$*(100/100 − Hct)	19 paired plasma and DBS samples were obtained from 12 patients, aged 2 mo to 18 y, treated with CBZ	Acceptable in Hct range of 30%-50%	FDA, ICH
50	One disc was extracted with 980 μL of MeOH:ACN (3:1, v/v) containing IS Sonication for 15 min	Overnight	-	-	Minimal effect of Hct range of 30%-55%	ICH
5	One disc was extracted with 100 μL of MeOH containing IS Vortex for 5 s	1	-	Fresh human BZD-free blood	-	EMA
5	One disc was extracted with 100 μL of MeOH containing IS Vortex for 5 s	1	Good correlation between C_p and C_{dbs} ($r^2 = 0.9841$)	Fresh human BZD-free blood	-	EMA
150	One disc was extracted with 1900 μL MeOH containing IS Vortex for 2 min Centrifuge at 3000 rpm for 10 min	0.25	Poor correlation between C_p and C_{dps} ($r = 0.903$)	96 samples of patients under anti-epileptic treatment	No evaluation about Hct effect due to the use of the DPS method	FDA
150	One disc was extracted with 1900 μL MeOH containing IS Vortex for 2 min Centrifuge at 3000 rpm for 10 min	0.25	-	-	No evaluation about Hct effect due to the use of the DPS method	FDA
25	Add 5 μL of IS. One disc was extracted by 25 μL acetic anhydride and 25 μL pyridine and subjected to microwave heating at maximum power for 90 s, followed by a second microwave-assisted derivatization for 90 s with 25 μL heptafluorobutanol. Short time centrifuge at 4℃.	2	$C_s = C_{dbs}/0.85$	Samples from 15 healthy volunteers aged 18-55 y with Hct range 0.37-0.48	Acceptable in Hct range of 30%-49%	FDA, ICH
20	One disc was extracted with MeOH 500 μL containing IS Vortex for 1 min Centrifuge for 10 min at 20 000 *g*	2	-	6 healthy volunteers	No evaluation about Hct effect due to the use of DPS method	FDA

(Continues)

TABLE 1 (Continued)

Medicine	Internal standard	Analytical method	Analytical range	Calibrator or QC DBS sampling method	Clinical DBS sampling method	Punch sizes (mm)	Paper type
Lamotrigine[37]	Lamotrigine 13C3, d3	LC-MS/MS	10-3020 ng/mL	Spiked plasma was spotted onto paper in 100 µL	The spiked plasma was spotted onto paper in 100 µL	15-mm paper cut into 4 pieces	Whatman 31 ET CHR papers chip
Lamotrigine[40]	Lamotrigine 13C3	LC-MS/MS	2.8-80 µmol/L	Spiked blood was spotted onto paper (30 µL)	Blood was spotted onto paper (30 µL)	4.7	Whatman 903 filter paper & protein saver card
Lamotrigine[41]	Lamotrigine-13C3	LC-MS/MS	0.25-40 µg/mL	Spiked EDTA blood was spotted onto paper (30 µL)	3-5 drops of blood were spotted onto paper by finger prick	3	Whatman 903 Protein Saver cards
Lamotrigine[46]	Linezolid	HPLC	0.6-24 µg/mL	Spiked plasma was spotted onto paper (150 µL)	Plasma was spotted onto paper (150 µL)	-	Dried sample spot devices (DSSD) from Laboratori Biomicron
Lamotrigine[25]	Lamotrigine-13C3	LC-MS/MS	0.25-40 µg/mL	Spiked EDTA blood was spotted onto paper (30 µL)	-	3	Whatman 903 filter paper
Lamotrigine[49]	Metformin	LC-UV	0.5-20 µg/mL	Spiked EDTA blood was spotted onto paper (10 µL)	EDTA blood was spotted onto paper (10 µL)	6	Whatman 903 Protein Saver cards
Lamotrigine[34]	Hexobarbital	HPLC	1-20 µg/mL	Spiked blood was spotted onto paper (30 µL)	-	6	Schleicher and Schuell 903 Guthrie cards
Levetiracetam[40]	Levetiracetam-d6	LC-MS/MS	5-400 µmol/L	Spiked blood was spotted onto paper (30 µL)	Blood was spotted onto paper (30 µL)	4.7	Whatman 903 filter paper and protein saver card

Sample volume (μL)	DBS sample extraction method	Dry time (h)	Comparison between DBS and plasma	Clinical sample	Hct effect	Method validation
25	Add 50 μL of IS and cut into four equal pieces Extract one piece with 2 mL of diethyl ether-methyl tert-butyl ether-acetone (50:30:20, v/v/v) Vortex for 20 min Centrifuge at 2690 *g* for 2 min	2	C_{dbs} and C_p could be interchangeable	Plasma samples were obtained from a two-way crossover bioequivalence study carried out in 14 healthy human volunteers (50 DPS sample)	No evaluation about Hct effect due to the use of the DPS method	FDA
30	One disc was extracted with 200 μL of MeOH/water (65:35, v/v) containing IS Microplate shaker at 450 rpm for 30 min	3	15% higher in DBS. ($r^2 = 0.9532$, $y = 1.0852x + 1.3489$)	Leftover blood from routine samples from patients on CBZ, LTG, LEV and VPA was used to produce DBS samples from venous or capillary blood	Acceptable in Hct range of 30%-50%	EMA
-	One disc was extracted with 200 μL of MeOH/water (80:20, v/v) containing IS Orbital shaker at 570 rpm for 5 min Centrifuge at 2100 *g* for 5 min	3	C_{dbs} could be changed to C_p without conversion	46 neuropaediatric patients aged 2-18 y with a mean age of 9 y on CBZ, LTG, or VPA treatment or on combination therapy	Acceptable in Hct range of 30%-55%	EMA
150	One disc was extracted with 1900 μL MeOH containing IS Vortex for 2 min Centrifuge at 3000 rpm for 10 min	0.25	Acceptable correlation between C_p and C_{dps} ($r = 0.989$)	Patients under anti-epileptic treatment	No evaluation about Hct effect due to the use of the DPS method	FDA
30	One disc was extracted with 200 μL of MeOH/water (80:20, v/v) containing IS Orbital shaker at 570 rpm for 5 min Centrifuge at 2100 *g* for 5 min	3	-	Healthy volunteers	Acceptable in Hct range of 30%-60%.	EMA
10	One disc was extracted with 500 μL ethyl acetate, 20 μL of 1 mol/L NaOH and 50 μL IS Vortex for 3 min, Centrifuge at 4500 *g* for 5 min	Overnight	Slightly higher results in DBS but these differences are not significant	12 samples from 4 different volunteers	Acceptable in Hct range of 25%-58%	FDA
50	One disc was extracted with 980 μL of MeOH:ACN (3:1, v/v) containing IS Sonication for 15 min	Overnight	-	-	Minimal effect of Hct range of 30%-55%	ICH
30	One disc was extracted with 200 μL of MeOH/water (65:35, v/v) containing IS Microplate shaker at 450 rpm for 30 min	3	C_{dbs} was comparable to Cp. ($r^2 = 0.9927$, $y = 0.9329x + 5.5571$)	Leftover blood from routine samples from patients	Acceptable in Hct range of 30%-50%	EMA

(Continues)

TABLE 1 (Continued)

Medicine	Internal standard	Analytical method	Analytical range	Calibrator or QC DBS sampling method	Clinical DBS sampling method	Punch sizes (mm)	Paper type
Levetiracetam[46]	Linezolid	HPLC	2.4-96 μg/mL	Spiked plasma was spotted onto paper (150 μL)	Plasma was spotted onto paper (150 μL)	-	Dried sample spot devices (DSSD) from Laboratori Biomicron
Levetiracetam[34]	Hexobarbital	HPLC	2-50 μg/mL	Spiked blood was spotted onto paper (30 μL)	-	6	Schleicher and Schuell 903 Guthrie cards
Monohydroxy carbamazepine[46]	Linezolid	HPLC	2.4-96 μg/mL	Spiked plasma was spotted onto paper (150 μL)	Plasma was spotted onto paper (150 μL)	-	Dried sample spot devices (DSSD) from Laboratori Biomicron
Nitrazepam[53]	Nordiazepam-d5	LC-MS/MS	20-5000 ng/mL	Spiked blood was spotted onto paper (5 μL)	Blood was spotted onto paper (5 μL)	6	Whatman 903 Protein Saver cards
Phenobarbital[34]	Hexobarbital	HPLC	2-50 μg/mL	Spiked blood was spotted onto paper in 30 μL	-	6	Schleicher and Schuell 903 Guthrie cards
Phenobarbital[54]	Phenobarbital-D5	LC-MS/MS	0-100 mg/L	Spiked pooled mixture of blood was spotted onto paper (20 μL)	Blood was spotted onto paper (20 μL)	3.2	Whatman 903 filter paper
Phenytoin[28]	5-Methylphenyl hydantoin	GC-MS	0.5-120 μg/mL	Spiked blood was spotted onto paper (30 μL)	2 drops of blood (~30 μL each) were spotted onto paper	6	Whatman 903 Protein Saver cards
Phenytoin[45]	-	LC-MS/MS	0-100 mg/L	Spiked blood was spotted onto paper (20 μL)	Blood was spotted onto paper (20 μL)	3.2	Whatman 903 filter paper
Phenytoin[51]	-	Substrate-labelled fluorescent immunoassay	-	-	Capillary blood was spotted onto paper	6	Whatman grade 160 filter paper, PKU-31 paper

Sample volume (μL)	DBS sample extraction method	Dry time (h)	Comparison between DBS and plasma	Clinical sample	Hct effect	Method validation
150	One disc was extracted with 1900 μL MeOH containing IS Vortex for 2 min. Centrifuge at 3000 rpm for 10 min	0.25	Fair correlation between C_p and C_{dps} ($r = 0.971$)	Patients under anti-epileptic treatment	No evaluation about Hct effect due to the use of the DPS method	FDA
50	One disc was extracted with 980 μL of MeOH:ACN (3:1, v/v) containing IS Sonication for 15 min	Overnight	-	-	Minimal effect of Hct range of 30%-55%	ICH
150	One disc was extracted with 1900 μL MeOH containing IS Vortex for 2 min. Centrifuge at 3000 rpm for 10 min	0.25	Acceptable correlation between C_p and C_{dps} ($r = 0.981$)	Patients under anti-epileptic treatment	No evaluation about Hct effect due to the use of the DPS method	FDA
5	One disc was extracted with 100 μL of MeOH containing IS Vortex for 5 s	1	Good correlation between C_p and C_{dbs} ($r^2 = 0.9841$)	Fresh human BZD-free blood	-	EMA
50	One disc was extracted with 980 μL of MeOH:ACN (3:1, v/v) containing IS Sonication for 15 min	Overnight	-	-	Minimal effect of Hct range of 30%-55%	ICH
20	Two discs were extracted with 200 μL of water/MeOH (20/80) solution containing IS Orbital shaker at 37°C for 20 min	2-3	Good correlation between C_p and C_{dbs} ($r^2 = 0.9953$) $C_p = C_{dbs}$*100/(100-Hct)	50 DBS from healthy controls and 50 spots from PWE whose phenobarbital levels had also been monitored	-	-
60	One disc was extracted with 500 μL of ACN and 1 molar sodium hydroxide (24:1, v/v) containing IS Vortex for 1 min Sonication for 5 min Centrifuge for 15 min at 6000 *g*	3	$C_p = \{1.11 * C_{dbs}/[1 - (0.71*Hct)] - 1.00$ μg/mL	165 PWE who were on either CBZ, VPA or/and PHT were recruited from October 2011 to August 2012 at a neurology specialist clinic of a tertiary referral hospital	-	FDA
20	One disc was extracted with 330 μL of MeOH/water (80/20) + formic acid (0.1%) Orbital shaker for 25 min at 37℃	-	High correlation between log-transformed C_p and C_{dbs} ($r^2 = 0.9821$)	Both plasma and whole blood specimens were collected from 17 paediatric patients	-	ICH
-	One disc was extracted with 490 μL of aqueous 5'-sulphosalicylic acid 50 g/L Agitation for 4 h Centrifuge at 1000 *g* for 5 min	-	High correlation between C_p and C_{dbs} ($r^2 = 0.9821$)	56 adults (25 male, 28 female) and 3 paediatric (male) unselected PWE receiving conventional single or combination anticonvulsant therapy	A tendency for the DBS-phenytoin concentration to fall with increasing Hct was demonstrated	-

(Continues)

TABLE 1 (Continued)

Medicine	Internal standard	Analytical method	Analytical range	Calibrator or QC DBS sampling method	Clinical DBS sampling method	Punch sizes (mm)	Paper type
Pregabalin[36]	Pregabalin-d4	LC-MS/MS	10-10 000 ng/mL	Spiked plasma was spotted onto paper (25 μL)	Plasma was spotted onto paper (25 μL)	15-mm paper cut into 4 pieces	Whatman 31 ET CHR papers chip
Pregabalin[55]	4-Aminocyclohexanecarboxylic acid	LC-MS/MS	0.200-20.0 μg/mL for DBS and 0.400-40.0 μg/mL for DPS	The spiked blood and plasma were spotted onto paper (50 and 40 μL, respectively)	EDTA blood and plasma were spotted onto paper in 50 and 40 μL, respectively	4	Whatman 903 filter paper
Rufinamide[46]	Linezolid	HPLC	2.4-96 μg/mL	Spiked plasma was spotted onto paper (150 μL)	Plasma was spotted onto paper (150 μL)	-	Dried sample spot devices (DSSD) from Laboratori Biomicron
Rufinamide[48]	-	LC-MS/MS	0-47.60 mg/L	Spiked pooled mixture of blood was spotted onto paper (20 μL)	Blood was spotted onto paper (20 μL)	3.2	Whatman 903 filter paper
Topiramate[32]	Topiramate-d12	GC-MS	5-300 μg/mL	The spiked blood was spotted onto paper (50 μL)	Blood was spotted onto paper (50 μL)	8	Whatman 903 filter paper
Topiramate[50]	Topiramate-d12	LC-MS/MS	0-50 mg/L	Spiked pooled mixture of blood was spotted onto paper (20 μL)	Blood was spotted onto paper (20 μL)	3.2	Whatman 903 filter paper
Topiramate [52]	Topiramate-d12	HPLC-MS/MS	10-2000 ng/mL	The spiked drug-free EDTA blood was spotted onto paper (30 μL)	Blood was spotted onto paper (30 μL)	6	FTATM DMPK-C DBS cards
Valproic acid[28]	5-Methylphenylhydantoin	GC-MS	0.5-120 μg/mL	Spiked blood was spotted onto paper (30 μL)	Two drops of blood (~30 μL each) were spotted onto paper	6	Whatman 903 Protein Saver cards

Sample volume (μL)	DBS sample extraction method	Dry time (h)	Comparison between DBS and plasma	Clinical sample	Hct effect	Method validation
6.25	Add 50 μL of IS and cut into four equal pieces The one piece was extracted with 3 mL methyl tert-butyl ether and diethyl ether (80/20, vol/vol) Agitation at 5 g for 5 min	2	C_{dbs} and C_p could be interchangeable	The newly developed method was applied for the quantification of pregabalin in plasma samples obtained from a 2-way crossover bioequivalence study performed in 14 healthy volunteers	No evaluation about Hct effect due to the use of the DPS method.	FDA
50 μL for DBS and 40 μL for DPS	Add 100 μL of IS The one disc was extracted with 500 μL of ethyl acetate Vortex for 10 min	2-3	-	12 PWE of different age, gender, body mass, neurological, cognitive, psychiatric and somatic status with or without co-medication	-	EMA
150	One disc was extracted with 1900 μL MeOH containing IS Vortex for 2 min Centrifuge at 3000 rpm for 10 min	0.25	-	-	No evaluation about Hct effect due to the use of the DPS method	FDA
20	One disc was extracted with 200 μL of water/ACN (30/70) + 0.05% of formic acid solution Orbital shaker at 37°C for 25 min	-	Good correlation between C_p and C_{dbs} ($r^2 = 0.9815$) $C_p = C_{dbs} \times (100/100 - Hct)$	10 DBS from healthy controls and 16 spots from 14 patients with confirmed epilepsy to whom rufinamide was administered	-	-
50	One disc was extracted with 300 μL of ACN and MeOH (1:3) containing IS Agitation for 30 min at 500 rpm, 45°C	-	The correlation between plasma and DBS concentrations was poor ($r = 0.61$)	DBS samples obtained from venipuncture and finger pricks from an adult individual	-	-
20	Two 3.2 mm discs were extracted with 200 μL of water/ACN (30/70) + 0.05% of formic acid solution containing IS Orbital shaker at 37°C for 20 min	-	TPM measurements from DBS to plasma concentration multiplying by 2.22 and by 1.79 for newborns (up to 1 mo of life) and adults, respectively	20 dried blood spots from healthy controls and 21 spots from PWE	-	-
30 μL	One disc was extracted with 200 μL of MeOH-water (90:10, v/v) containing IS Vortex for 10 min Centrifuge for 5 min at 3000 *g*	3	-	-	Acceptable in Hct range of 34%-45%	FDA, EMA
60	One disc was extracted with 500 μL of ACN and 1 molar sodium hydroxide (24:1, v/v) containing IS Vortex for 1 min Sonication for 5 min Centrifuge for 15 min at 6000 *g*	3	$C_p = \{0.92 * C_{dbs}/[1 - (0.96*Hct)]\} - 12.48$ μg/mL	165 PWE who were on either CBZ, VPA or PHT were recruited from October 2011 to August 2012 at neurology specialist clinic of a tertiary referral hospital	-	FDA

(Continues)

TABLE 1 (Continued)

Medicine	Internal standard	Analytical method	Analytical range	Calibrator or QC DBS sampling method	Clinical DBS sampling method	Punch sizes (mm)	Paper type
Valproic acid[38]	Cyclohexane carboxylic acid	GC-MS	5-250 μg/mL	Spiked blood was spotted onto paper (50 μL)	A drop of blood was spotted onto paper by finger prick	6	Whatman 903 filter paper
Valproic acid[40]	Valproic acid-d6	LC-MS/MS	20-1000 μmol/L	Spiked blood was spotted onto paper (30 μL)	Blood was spotted onto paper (30 μL)	4.7	Whatman 903 filter paper and protein saver card
Valproic acid[41]	Valproic acid-d6	LC-MS/MS	5-300 μg/mL	Spiked EDTA blood was spotted onto paper (30 μL)	3-5 drops of blood were spotted onto paper by finger prick	3	Whatman 903 Protein Saver cards
Valproic acid[25]	Valproic acid-d6	LC-MS/MS	5-300 μg/mL	Spiked EDTA blood was spotted onto paper (30 μL)	-	3	Whatman 903 filter paper
Valproic acid[47]	-	HPLC	10-1200 μmol/L	Spiked blood was spotted onto paper (20 μL)	Venous blood was spotted onto paper (20 μL)	5 or 6	Whatman 903 filter paper
Valproic acid[24]	Valproic acid-d6	GC-MS	10-200 μg/mL	Spiked plasma was spotted onto paper (20 μL)	Plasma was spotted onto paper (20 μL)	8	Whatman FTA DMPK-A cards or Bond Elut DMS card
Vigabatrin[56]	4-Aminocyclohexane carboxylic acid	LC-MS/MS	0.5-50 μg/mL	Spiked plasma was spotted onto paper (40 μL)	Plasma was spotted onto paper (40 μL)	5	Whatman 903 filter paper

Sample volume (µL)	DBS sample extraction method	Dry time (h)	Comparison between DBS and plasma	Clinical sample	Hct effect	Method validation
50	The one disc was extracted with 200 µL extraction solution (mixture of ACN and MeOH (1:3, v/v), containing IS) Ultrasonication for 1 h	3	High correlation between serum and DBS (r = 0.9948) Cs = 1.883*Cdbs	The DBS assay method was applied to 17 samples collected from September to October 2013 from patients	Acceptable in Hct range of 30%-50%	-
30	One disc was extracted with 200 µL of MeOH/water (65:35, v/v) containing IS Microplate shaker at 450 rpm for 30 min	3	35% lower in DBS. (r^2 = 0.9488, y = 0.6955x-16.107)	Leftover blood from routine samples from patients on CBZ, LTG, LEV and VPA was used to produce DBS samples from venous or capillary blood	Acceptable in Hct range of 35%-50%	EMA
-	One disc was extracted with 200 µL of MeOH/water (80:20, v/v) containing IS Orbital shaker at 570 rpm for 5 min Centrifuge at 2100 *g* for 5 min	3	$C_p = 1.58*C_{dbs}$	46 neuropaediatric patients aged 2-18 y	Acceptable in Hct range of 30%-55%	EMA
30	One disc was extracted with 200 µL of MeOH/water (80:20, v/v) containing IS Orbital shaker at 570 rpm for 5 min Centrifuge at 2100 *g* for 5 min	3	-	Healthy volunteers	Acceptable in Hct range of 35%-60%	EMA
20	The one disc was extracted with 150 µL extraction solution (MeOH containing internal standard) Agitation for 60 min Centrifuge at 2100 *g* for 5 min	Overnight	27% lower in DBS method (r^2 = 0.9)	34 venous blood samples from anonymized patients treated with valproic acid	Acceptable in Hct range of 30%-60%	EMA
20	One disc was extracted with MeOH 500 µL containing IS Vortex for 1 min Centrifuge for 10 min at 20 000 *g*	2	-	6 healthy volunteers	No evaluation about Hct effect due to the use of the DPS method.	FDA
40	One disc was extracted with 100 µL of working IS solution Vortex for 10 min	2-3	-	12 PWE or West syndrome received vigabatrin without co-medication	No evaluation about Hct effect due to the use of the DPS method	FDA, EMA

(Continues)

TABLE 1 (Continued)

Medicine	Internal standard	Analytical method	Analytical range	Calibrator or QC DBS sampling method	Clinical DBS sampling method	Punch sizes (mm)	Paper type
Zonisamide[46]	Linezolid	HPLC	2.4-96 μg/mL	Spiked plasma was spotted onto paper (150 μL)	Plasma was spotted onto paper (150 μL)	-	Dried sample spot devices (DSSD) from Laboratori Biomicron

Abbreviations: AED, anti-epileptic drugs; BZD, benzodiazepine; CBZ, carbamazepine; C_p, concentration of plasma; C_{dbs}, concentration of dried blood spot; C_s, concentration of serum; DBS, dried blood spot; DPS, dried plasma spot; EDTA, ethylenediaminetetraacetic acid; EMA, European Medicines Agency; FDA, US Food and Drug Administration; GC-MS, gas chromatography-mass spectrometry; Hct, haematocrit; HPLC, high-performance liquid chromatography; ICH, The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; IS, internal standard; LC-MS/MS, liquid chromatography with triple quadrupole mass spectrometer; LC-UV, liquid chromatography with ultraviolet detection; LEV, levetiracetam; LTG, lamotrigine; PHT, phenytoin; PWE, patients with epilepsy; QC, quality control; TPM, topiramate; VPA, valproic acid.

do not undergo syringe sampling in hospital. However, the final concentration for analysis can be severely affected by insufficient drying, contamination by the external environment, or a small sample volume. To address these limitations, DBS paper could be treated with desiccants or antimicrobial agents, or patients could be taught how to collect specimens for the DBS method.[9] Analysis of DBS samples is simple and takes less time. In general, there is no need for centrifugation and plasma extraction to analyse drug concentrations. Convenient analysis is possible because blood cells remain fixed to the paper, while only drug molecules are released.[33]

That being said, the DBS method also has several limitations. Each drug requires a database to be established. Conventional target drug concentrations are based on plasma; therefore, results may be different from those obtained from DBS samples, which need to undergo a drying process for a defined period of time and include blood cells and plasma. Therefore, it is necessary to identify the drug concentration correlation between the venous blood and the capillary blood obtained by finger prick. In addition, the PK characteristics of each drug should be evaluated, and data on factors such as storage temperature, analysis period, target concentration range and stability in DBS are required.[7,34] Another limitation is the very sensitive and expensive equipment currently used for DBS analysis, such as that required for chromatography and mass spectrometry.[33,35] In addition, very small amounts of the specimen are required; therefore, errors are likely to occur when simultaneously analysing drugs with a wide concentration range.

6 | AED CONCENTRATION MEASUREMENT USING DBS OR DPS

Studies conducted from 1984 to 2018 on blood concentration measurements of AEDs using the DBS method were reviewed. Information about internal standards, analytical methods and ranges, sampling methods, punch sizes, paper types, sample and injection volumes, drying times, DBS sample extraction methods, effects of Hct levels and results of comparisons between DBS and plasma were summarized (Table 1). In 26 studies, 19 types of AEDs were measured using the DBS method [24,25,28,32,34,36-56]; among them, 9 studies analysed >2 AEDs.[24,25,28,34,40,41,44,46,53] The most frequently analysed AED was carbamazepine (n = 8); others included lamotrigine (n = 7), valproic acid (n = 7), levetiracetam (n = 3), phenytoin (n = 3), topiramate (n = 3), carbamazepine epoxide (n = 2), gabapentin (n = 2), phenobarbital (n = 2), pregabalin (n = 2), clobazam (n = 1), clonazepam (n = 1), ethosuximide (n = 1), felbamate (n = 1), monohydroxycarbamazepine (n = 1), nitrazepam (n = 1), rufinamide (n = 1), vigabatrin (n = 1) and zonisamide (n = 1). Various analytical methods can be used to quantify the concentration of AEDs in DBS or DPS samples. LC-MS/MS (n = 15) was most frequently used; other analytical methods included gas chromatography-mass spectrometry (n = 6), high-performance liquid chromatography (n = 5) and fluorescent immunoassay (n = 1). Currently, LC-MS/MS is predominantly used for analysing DBS samples because of its high sensitivity and selectivity.[57] Most analytical methods reported in the included studies were validated by the FDA, European Medicines Agency and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines.

Several studies demonstrated a relationship between the C_{dbs} and C_p for carbamazepine. Moreover, previous studies reported a consistent tendency for C_{dbs} to be higher, such that it could be converted to C_p. Two studies compared C_{dbs} and C_p of carbamazepine in patients with epilepsy to derive direct conversion formulae: Kong et al[28] recommended $C_p = (0.89 \times C_{dbs}) + 1.00$ μg/mL and Linder et al[41] recommended $C_p = 0.84 \times C_{dbs}$. In contrast, Shokry et al[44] suggested that C_{dbs} was highly correlated with C_p after adjusting for Hct levels in paediatric patients ($r^2 = 0.9502$).

Sample volume (μL)	DBS sample extraction method	Dry time (h)	Comparison between DBS and plasma	Clinical sample	Hct effect	Method validation
150	One disc was extracted with 1900 μL MeOH containing IS Vortex for 2 min Centrifuge at1500 *g* for 10 min	-0.25	-	-	No evaluation about Hct effect due to the use of the DPS method	FDA

The only PK study that used DBS sampling evaluated carbamazepine; the results demonstrated that total daily dose and gender were covariate factors of C_p, whereas total daily dose was the only factor of C_{dbs}.[39] This suggests that C_{dbs} and C_p could be substituted in PK studies without any difference. The serum concentration (C_s) and C_p of carbamazepine also demonstrated similar tendencies. One study comparing the C_s and C_{dbs} of carbamazepine among 80 patients with epilepsy [42] found that Hct levels had no effect and derived the following conversion formula: $C_s = C_{dbs} \times 0.83 + 1.09$. One study of carbamazepine epoxide, which is the active metabolite of carbamazepine and has comparable activity, demonstrates that C_{dbs} was correlated with C_p after adjusting for Hct levels ($r^2 = 0.9493$).[58] Clear relationships were demonstrated between C_{dbs} and C_p of both carbamazepine and carbamazepine epoxide. The conversion equations were also concise and precise in both adults and children. These findings suggest that the DBS method could soon be applicable to TDM.

Studies about benzodiazepines, including clobazam, clonazepam and nitrazepam, demonstrated good correlations between the C_{dbs} and C_p ($r^2 = 0.9847$).[53] However, the calibration curve of each of these AEDs was not assessed. Moreover, only single samples of clonazepam and nitrazepam obtained from humans were included for clinical validation.

Ethosuximide and felbamate were simultaneously measured using dried sample spot devices.[46] However, the correlation between C_p and the concentration in DPS (C_{dps}) for ethosuximide was poor ($r = 0.903$), and clinical validation of felbamate was not conducted. On the basis of this study, the DPS method would be difficult to use for ethosuximide; in addition, no study has measured ethosuximide and felbamate using the DBS method. Therefore, problems remain when applying ethosuximide and felbamate in clinical practice, and further research should be conducted.

Sadones et al attempted to identify a relationship between C_s and C_{dbs} for gabapentin. They determined an easy conversion formula ($C_s = C_{dbs}/0.85$) and demonstrated a good correlation between C_s and C_{dbs} ($r = 0.9353$). However, this could only be applied in participants with an Hct level of 30%-49%.[43] One interesting study of gabapentin by Ikeda et al[24] compared outcomes using Whatman® FTA DMPK-A® and Bond Elut DMS® paper; both demonstrated acceptable precision and accuracy using the DPS method, which ranged from 0.5 to 10 μg/mL (calibration curve $r^2 > 0.99$). However, the sample was unstable at 30°C; therefore, storage conditions below this temperature would enable the clinical application of gabapentin.

Lamotrigine was assessed in seven studies; among these, one study did not compare DBS and plasma,[25] two used DPS [37,46] and four used DBS.[34,40,41,49] Results of the studies that used the DPS method demonstrated that C_{dps} could be converted to C_p without any changes. In the studies of adults that used the DBS method, C_{dbs} was slightly higher than C_p.[40,49] Linder et al[40] found that C_p from venous and capillary blood was well correlated with C_{dbs} ($r^2 = 0.9532$), and C_p was 15% lower than C_{dbs} within an Hct level of 30%-50%. In addition, Aburuz et al[49] reported that C_p was slightly lower (2%-7%) than C_{dbs}; however, there was no significant difference. One study developed the DBS analysis method using paediatric blood and found a good correlation between C_{dbs} and C_p with minimal effect when the Hct level ranged from 30% to 55% ($r > 0.995$).[34] Another study of paediatric patients with epilepsy, which was clinically validated, found that C_{dbs} was slightly higher than C_p and could be used for TDM without adjusting for Hct levels.[41]

Levetiracetam, a recently developed second-generation AED, had good efficacy and safety in paediatric patients with epilepsy.[59-62] In addition, it was not metabolized by cytochrome P450 and has an extremely low protein-binding

TABLE 2 Development status of anti-epileptic drugs

	DPS or DBS method development	Comparison between plasma and DPS	Comparison between serum and DBS	Comparison between plasma and DBS	Finger prick	In paediatric patients	Population modelling
Carbamazepine	DBS	X	O	O	O	O	O
Carbamazepine epoxide	DBS	X	X	O	X	O	X
Clobazam	DBS	X	X	X	X	X	X
Clonazepam	DBS	X	X	O	X	X	X
Ethosuximide	DPS	O	X	X	X	X	X
Felbamate	DPS	X	X	X	X	X	X
Gabapentin	Both	X	O	X	X	X	X
Lamotrigine	Both	O	X	O	O	O	X
Levetiracetam	Both	O	X	O	X	X	X
Monohydroxycarbamazepine	DPS	O	X	X	X	X	X
Nitrazepam	DBS	X	X	O	X	X	X
Phenobarbital	DBS	X	X	O	X	X	X
Phenytoin	DBS	X	X	O	X	O	X
Pregabalin	Both	O	X	X	X	O	X
Rufinamide	Both	O	X	O	X	O	X
Topiramate	DBS	X	X	O	O	O	X
Valproic acid	Both	X	O	O	O	O	X
Vigabatrin	DPS	X	X	X	X	O	X
Zonisamide	DPS	X	X	X	X	X	X
Primidone	X	X	X	X	X	X	X
Oxcarbazepine	X	X	X	X	X	X	X
Stiripentol	X	X	X	X	X	X	X
Tiagabine	X	X	X	X	X	X	X
Lacosamide	X	X	X	X	X	X	X
Eslicarbazepine	X	X	X	X	X	X	X
Retigabine	X	X	X	X	X	X	X
Perampanel	X	X	X	X	X	X	X

Abbreviations: DBS, dried blood spot; DPS, dried plasma spot.

affinity. Therefore, it has appropriate characteristics for application to the DBS method.[63] An analytical method using DPS sampling was developed and demonstrated a fair result ($r = 0.971$) [46]; other methods that used DBS sampling also demonstrated a good correlation between C_{dbs} and C_p ($r > 0.995$).[34] A recent clinical validation involving 15 patients with epilepsy found that C_{dbs} was comparable with C_p within an Hct level of 30%-50% ($r^2 = 0.9927$).[40] Further validation among a larger sample of patients with epilepsy is required before its application in clinical practice and TDM.

Only one study that used the DPS method evaluated monohydroxycarbamazepine, which is an active metabolite of oxcarbazepine.[46] C_{dps} and C_p demonstrated a good correlation ($r = 0.981$). However, this study included a small patient sample and did not evaluate oxcarbazepine and monohydroxycarbamazepine together.

Phenobarbital is among the oldest AEDs; however, it has been evaluated in only one study using the DBS method. The C_{dbs}, adjusted for Hct level, demonstrated good agreement with C_p in healthy volunteers ($r^2 = 0.9953$).[54] Recent studies of phenobarbital have not been conducted because of its declining use and unsuitable PK properties for DBS sampling.

Phenytoin was the earliest AED to be analysed using the DBS method in 1984. In that study, capillary blood for DBS analysis and venous blood were obtained simultaneously.[51] A good correlation was found between the C_{dbs} and C_p ($r = 0.9889$) and the C_{dbs} decreased as Hct level increased. In another study, the C_p of phenytoin was 2.8 ± 1.89 μg/mL higher than C_{dbs}. On the basis of this result, Kong et al[28] developed the following estimation equation: $C_p = (1.11 \times C_{dbs}/[1-(0.71 \times Hct)]) - 1.00$ μg/mL. Villanelli et al[45] reported that C_{dbs} was highly correlated with C_p after adjusting for Hct levels, which is consistent with findings of other studies ($r^2 = 0.982$). However, C_p and C_{dbs} were log-transformed when compared.

Only one bioequivalence study in healthy volunteers compared the C_{dps} and C_p of pregabalin and found that C_{dps} could be converted to C_p without introducing any statistical bias.[36] Another study compared C_{dps} and C_{dbs} after adjustment for Hct levels and found a high correlation ($r^2 = 0.977$).[55] More studies should be conducted to identify the relationship between C_{dbs} and C_p.

The C_p and C_{dbs} of rufinamide were compared in one study by La Marca et al[48] A good correlation was demonstrated after adjusting for Hct ($r^2 = 0.9815$), and the following formula was suggested as follows: $C_p = C_{dbs} \times (100/100 - Hct)$.

La Marca et al[50] developed a simple method to convert C_{dbs} to C_p for topiramate. For newborns and adults, the simple calculation was $C_p = C_{dbs} \times 2.22$ and $C_p = C_{dbs} \times 1.79$, respectively. Another study monitored the PK of 100 mg topiramate for 96 hours after dosing.[32] The estimated fraction in plasma ($F_p = C_p/(C_{dbs}/(1 - Hct))$) was correlated only between 0.5 and 24 hours; therefore, conversion from C_{dbs} to C_p was difficult and only possible within a limited concentration range.

Valproic acid was assessed in several studies that demonstrated a similar tendency for C_p to be higher than C_{dbs}. Pohanka et al[47] reported that C_p of valproic acid was 27% higher than C_{dbs}, and higher Hct levels had a greater impact. In another study involving 13 patients with epilepsy, C_{dbs} of valproic acid was 35% higher than C_p.[40] Kong et al[28] developed the following equation to estimate C_p from C_{dbs} using the Hct level and ratio of red blood cells to plasma: $C_p = (0.92 \times C_{dbs}/[1 - (0.96 \times Hct)]) - 12.48$ μg/mL. In a study involving paediatric patients, C_p was calculated after adjusting for Hct simply as $C_p = C_{dbs} \times 1.53$.[41] The relationship between the C_s and C_{dbs} of valproic acid obtained from finger-prick samples demonstrated a strong correlation ($r = 0.9948$) and provided a simple conversion equation as follows[38]: $C_s = C_{dbs} \times 1.883$.

In the cases of vigabatrin and zonisamide, only the DPS method was developed using LC-MS/MS and high-performance liquid chromatography, without determining the relationship with C_p.[46,56]

7 | CLINICAL IMPLICATIONS

The DBS method can be used in paediatric patients because it is less invasive and requires only a small amount of blood compared with the traditional sampling methods; this is considered its greatest advantage.[13] Accordingly, the DBS method could reduce the repulsion of blood sampling in children.[12] To harness these benefits, a finger-prick method for use among paediatric patients should be established. However, only four studies compared C_{dbs} obtained from finger-prick sampling with C_p (or C_s),[32,38,41,49] and only one compared it with venous blood.[32] For each AED, the development of the DBS method was at different stages depending on the time of introduction and extent of use in the market (Table 2).

The most commonly studied AEDs using the DBS method were carbamazepine, lamotrigine, topiramate and valproic acid. These were assessed in adult and paediatric patients, and good correlations between their C_{dbs} and C_p obtained using the finger-prick method were demonstrated, enabling the conversion between the values. In particular, a precise conversion formula for carbamazepine was established by using population PK modelling.

Another drug with high potential for clinical application is phenytoin, which has undergone extensive evaluation, including validation of the relationship between C_p and C_{dbs} and clinical validation among paediatric patients. However, there have been no studies of finger-prick sampling. Phenytoin is a widely used drug in children; therefore, use of the DBS method could soon be available for TDM, and provided studies of finger-prick sampling are conducted.

Levetiracetam is a second-generation AED and also holds promise. Its advantages include lower protein-binding affinity, faster absorption, reduced drug-drug interaction profile, better tolerability and lower adverse events compared with those of first-generation AEDs. Evaluations of levetiracetam have been conducted as its usage has increased, and include clinical validation performed in adults. Therefore, provided clinical validations in paediatric patients and studies of finger-prick sampling are conducted; it is expected that the DBS method could soon be used for TDM of levetiracetam.

Other second-generation AEDs include gabapentin, pregabalin, felbamate, rufinamide, vigabatrin and zonisamide. Gabapentin and pregabalin are widely used for seizure and neuropathic pain control. However, TDM is not usually performed; therefore, the DBS method might not be well developed. The remaining drugs are not widely used and have been assessed in a few studies only. Therefore, DBS methods are unlikely to be developed unless the usage for these drugs increases.

Ethosuximide and phenobarbital are old first-generation AEDs but have been evaluated in a limited number of studies. Further studies would be difficult to conduct because their demand has decreased with the development of better AEDs. In addition, ethosuximide demonstrated unsatisfactory results using the DPS method despite having low protein-binding affinity. Therefore, TDM of these two drugs may be difficult to perform using DBS.

DBS methods for clobazam, clonazepam and nitrazepam were developed together with other benzodiazepine drugs. However, clobazam was not evaluated in human participants, and the C_{dbs} and C_p of clonazepam and nitrazepam were each compared using a single sample. In addition, their demand for TDM is low; therefore, progress is difficult.

With respect to the DBS method, other AEDs (primidone, oxcarbazepine, stiripentol, tiagabine, lacosamide, eslicarbazepine, retigabine and perampanel) were not assessed. These may have been developed relatively recently or may be used less frequently in clinical practice.

8 | CONCLUSIONS

Several AEDs have narrow therapeutic indices, which could contribute to the occurrence of drug-drug interactions. Therefore, TDM is widely used to optimize treatment in patients with epilepsy. Frequent blood sampling for TDM could be a burden in paediatric patients because venepuncture is invasive and hurtful; therefore, the DBS method provides a good alternative. It is less invasive and requires a small amount of blood compared with traditional sampling methods. This could contribute to better adherence among paediatric patients. To apply the DBS method in TDM, the development status, which is different for each AED, must be evaluated at several stages. We assessed the development status of available drugs and found that carbamazepine, lamotrigine, topiramate and valproic acid are available for TDM using the DBS method, whereas levetiracetam and phenytoin are promising treatments.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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