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Dried spot sample and its drug detection using LC-MS/MS: Trends and advances in matrix collection and bioanalytics

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ABSTRACT

Therapeutic drug monitoring involves the clinical study of the drug and its metabolite in a biological matrix amidst the existing endogenous substances. One of the key components in performing therapeutic drug monitoring is the biological matrix. The collection and storage of biological matrix have developed rapidly in the last few decades. Dried blood spot (DBS) is a method which was introduced in the 1960s and has gone through series of advanced till date. These involve use of filter paper or punch cards to harvest and store blood for further use. One of the major disadvantages of DBS is the influence of hematocrit values in drug collections therefore paving way for dried plasma spot detection (DPS). Use of DBS and DPS is a growing trend observed in the sample collection. In the last few years, many assays have been developed based on this technique this paper introduced the pross and cons of DBS and DPS while looking into the advances in the drug detection using these matrixes.

INTRODUCTION

Therapeutic drug monitoring involves qualitative and quantitative analysis of drug and their concentration in biological matrix (such as tissue, whole blood, plasma, serum, urine, and saliva) (Anderson *et al*., 2004; Kole *et al*., 2011; Navitha Reddy *et al*., 2019). The most predominant biological matrix used in therapeutic drug monitoring (TDM) is blood or plasma sample due to distribution of drugs via blood. Due to the extensive demand for the blood samples for TDM, alternative method of sample collection is essential, where studies can be carried out without extensively harvesting blood from patients and the volume blood required doubles when the study has to be performed in plasma sample.

When performing studies regarding drug concentration and distribution, sampling of blood plasma and whole blood is golden standards. Conventional method of sample collection in TDM is venous blood collection. Therefore, requires extraction of lots of blood, followed by centrifugation of the venous blood to separate plasma sample from them (Avataneo *et al*., 2019). This is not only time consuming but also increases unnecessary visits to phlebotomist.

The first reported case of using filter paper in the year 1963 by a group of researchers in Canada, where neonatal sample was collected by pricking finger and collecting it by blotting it on a filter paper for the detection of phenylketonuria in neonates (Guthrie and Susi, 1963) laying a strong foundation for dry blood spot collection method and its processing. This technique has been extensively studied in the last few decades (Fig. 1).

Dried blood spot (DBS) alleviates the amount of blood collected, therefore, easing the procedure as well as reducing pain due to invasive procedure of venous blood harvesting. Patients suffering from delirium as well as patients suffering from severe case of Trypanophobia could benefit from such simple minimal invasive technique.

DBS/dried plasma spot (DPS) requires patients to prick their finger with a sterile and disinfected lancet and spot the blood on an encircled area in a filter paper. This filter paper is dried at the room temperature. This is follows by packing them in a plastic pouch and is transported to laboratories for its studies. Once it reaches the laboratories, appropriate extraction/pre-validated extraction procedure is performed on the DBS is done to monitor drug concentration (Ates *et al*., 2020). It is a very convenient way

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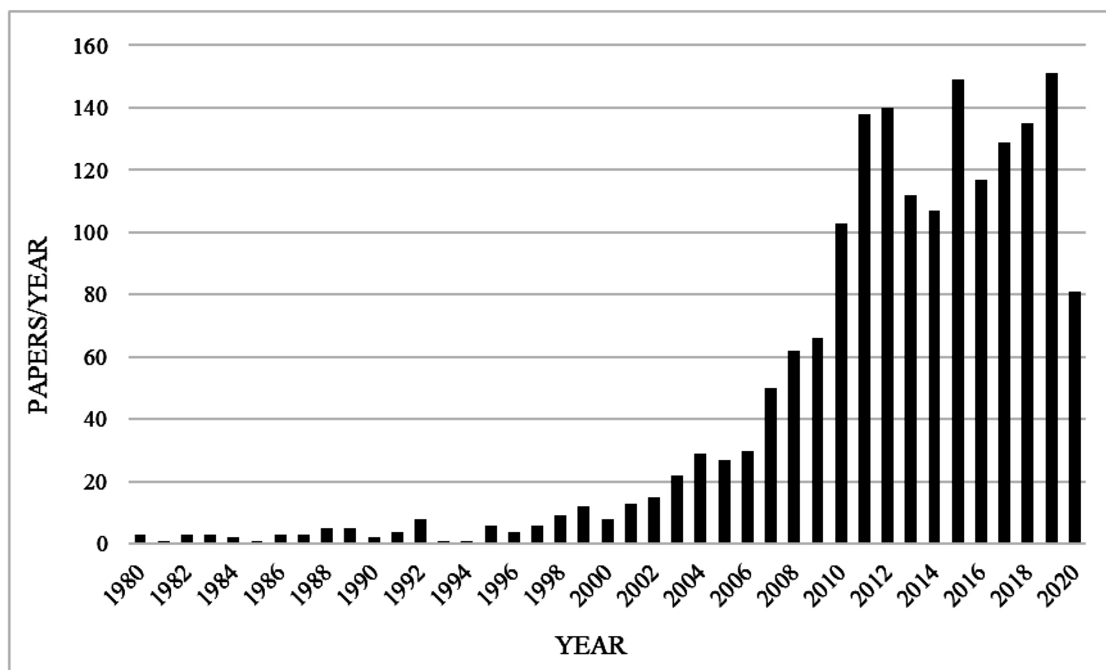

Figure 1. Number of paper published per year based on DBS/DPS in the last few years.

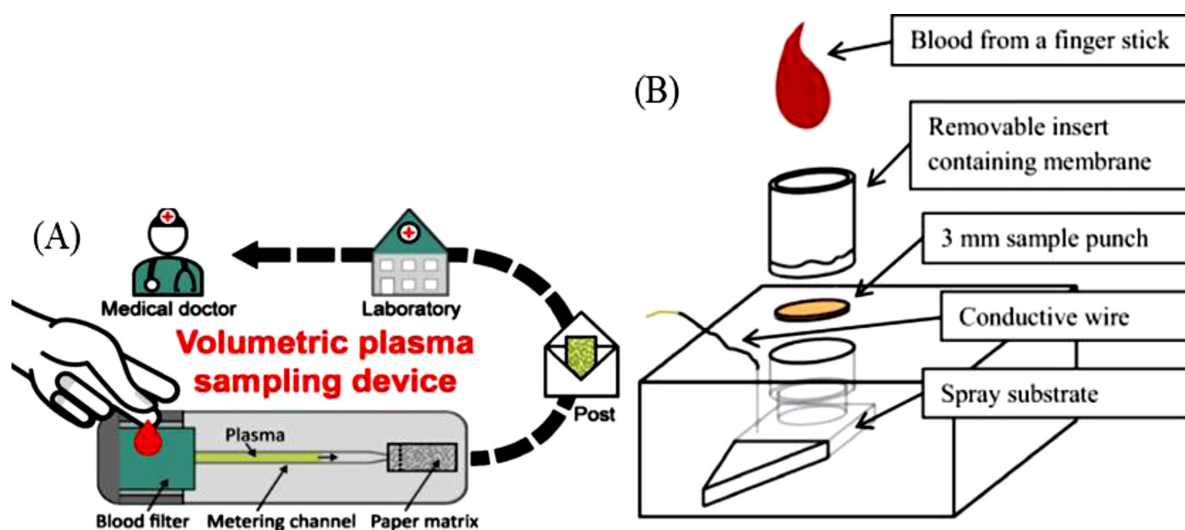

Figure 2. Plasma filtration methods. (A) Volumetric plasma extraction from whole blood (Hauser *et al*., 2019). (B) Blood fractionation cartridge with paper spray isonization set up (Bills *et al*., 2016)

of collection of samples which requires little to no expertise in the field of sample collection. The samples can be provided by patients without assistance.

Collection of plasma sample, however, involves centrifuging the DBS as soon as it is spotted. To avoid such inconvenience, many researchers have reported method which incorporate an inbuilt plasma filtration setup. One such example is a membrane substrate DPS card is developed and reported by Sturm *et al*. (2015). Another example is explained by Kim *et al*. (2013). The filtration-based membrane stacked set up to filter RBCs from the blood spot on the top layer followed by collection of plasma that is produced post filtration in the bottom layer (Kim *et al*., (2013).

Many other plasma separation microfluidic devices are also developed which are capable of separating required volume of plasma from the blood. One such device is reported by Hauser *et al*. (2019). This device was capable of having an input volume (40–80 µl), and separate plasma from them with varying hematocrit levels ranging from 39% to 45%. They further tested their device by detecting plasma level of caffeine. Another

example of separation of plasma from whole blood was reported by Bills and Manicke (2016). This paper reported the use of fractional separation principle for the separation of plasma from the blood (Fig. 2).

Basic Protocol of DBS/DPS

The most widely used extraction method for the extraction of the drug from the DBS/DPS is by protein precipitation method. This involves cutting out the spot and placing them in a vial. This is followed by addition of either methanol or acetonitrile as a precipitating agent. Pre-treatment/rehydration of the sample is usually not required.

As a part of pre-treatment, the spot is allowed to incubate in the precipitating agent. Then the sample is vortexed and centrifuged to separated filter paper fibers and possible endogenous substances from the sample. The supernatant is transferred and loaded to liquid chromatography/tandem mass spectrometry (LC-MS/MS) system for its detection. Modification in conventional protein precipitations is also employed to increase the recovery. Some of the modifications used include use of combination of protein precipitation and liquid-liquid extraction (LLE), microwave assisted protein precipitation, and ultrasound assisted protein precipitation to name few.

However, other conventional sample preparation methods such as LLE and solid phase extraction (SPE) are also employed but protein precipitation technique is preferred due to the ease of the method. Alternatively, modern sampling method include Automated Flow-through Spot Elution and On-line SPE, PPT-automated and the use of fully automated DBS-MS 500 system CAMAG.

Key Factors in LC-MS/MS Method Development Using DBS and DPS

Various factors have to be optimized in order to develop a method of detection using DBS/DPS. The factors include hematocrit effect, type of filter paper/DBS card, spot volume, carry over/Matric effect, correlation of DBS/DPS and plasma/ Circulating Blood Concentrations of drug under study, storage conditions, and sample preparation.

Type of filter paper

The thickness, density, nature of the filter paper (amount of cellulose content), and processing chemicals (presence of proteinase or other interfering content) can affect the extraction efficiency or the recovery of the drug from the spot (Cho *et al*., 2019). Some of the filter papers employed include Whatman-Protein Saver cards, Whatman 903, Ahlstrom 226, Whatman FTA drug metabolism pharmacokinetic (DMPK)-A/DMPK-B/DMPK-C, Whatman 31 ET CHR, Whatman 3, and Agilent Bond Elut DMS.

Each filter paper has specific properties. DMPK A and DMPK B have been treated with proteinases and result in denaturation of proteins as soon as blood is in contact with them. They are often used to denature proteins and lyse cells in the blood sample of plasma sample (Wilhelm *et al*., 2014). They are also used for micro sampling and pharmacokinetic (PK) studies. On the other hand, DMPK C filter paper is not treated with any chemicals hence have pure cellulose. DMPK C cards have been widely used for bioanalysis studies (Luckwell *et al*., 2014). However, Whatman 903 and Ahlstrom 226 have been widely employed for blood sample collection. Whatman 903 and Ahlstrom 226 have been recommended by US Food and Drug Administration as medical devices which can be used for collection of blood samples.

Due to the different properties of each paper, trials have to be taken to select the best filter paper which causes minimal effect on the recovery of the drug under the study. This ensures reliability as well as providing higher chances of increasing the recovery. Cho *et al*. (2019) evaluated the extraction efficiencies of trimethylamine *N*-oxide and eight related compounds in 903, Whatman 903 Proteinsaver card; DMPK-A, Whatman FTA DMPK-A card; DMPK-B, Whatman FTA DMPK-B card; DMPK-C, Whatman FTA DMPK-C card. They concluded that the recovery of trimethylamine *N*-oxide its related compounds. They reported that Whatman 903 (75.2%–92.3%), DMPK-A (65.6%–85.2%), and DMPK-C (50.3%–70.5%) showing that the difference in the properties of each filter paper greatly influence the recovery of the analyte.

Spot volume

Li *et al*. (2015) have studied that the spot volume greatly affects the concentration of the extracted drug from the plasma. In his studies, he studied two concentration-lower quality control and

Impact of plasma volume (µL) spotted on the accuracy of determination of ritonavir in DPS samples on DMPK C cards ($n = 4$).

Plasma volume spotted (µL)	Nominal conc. (ng/mL)	Mean measured (ng/mL)	CV (%)	% Diff
10	150	123	1.4	−20.6
20	150	138	2.7	−10.8
25	150	155	4.3	0.0
30	150	158	6.0	1.7
10	3750	3030	1.5	−20.7
20	3750	3560	1.9	−6.7
25	3750	3820	1.5	0.0
30	3750	3850	4.5	0.7

% Diff = ((Mean – 25 µL plasma spot mean)/25 µL plasma spot mean × 100).

Figure 3. Impact of plasma volume or spot volume e in drug detection and its recovery (Li *et al*., 2015).

higher quality control levels of ritonavir concentration he showed that there was considerable amount of concentration variation seen post extraction in these concentration as he changed the spot volume from −10 to −30 µl (Li *et al*., 2015) (Fig. 3).

Moat *et al.* (2020) studied the effect of blood volume on analytical bias in DBSs where he suggested that the higher the volume of blood spotted, greater is the volume of blood collected from the spot and therefore more analyte concentration and vice versa. However, beyond a certain range, in the lower limit if the volume of blood is low, then the chances of negative bias increases. They suggest the used of spot volume in the range of 25–75 µl or a spot diameter of 7–14 mm (Moat *et al*., 2020).

Correlation of DPS/DBS with plasma/blood concentration level

When performing/developing a DBS assay, it is known that the low volume of blood is require which is usually acquired through picking either finger or heal (in case of neonates). It is important to take into consideration that the prick blood is usually capillary blood which is a mixture of both arterial and venous blood. There may be significant difference in drug distribution in both the system post drug treatment. Therefore, evaluating the difference in these two systems is important to relate the two studies.

The pharmacokinetics and pharmacodynamics (PK/PD) of any drugs are closely related to the bound and unbound concentration of drug present in plasma of the circulating blood; hence, it becomes imperative to understand the fraction of bound, unbound and total plasma. It is reported that the red cell membrane acts as a barrier therefor hindering the free circulation of drug in the circulatory system. The following equation represents the relation of the unbound, bound and total plasma drug concentration at equilibrium considering no drug is degraded.

$$C = \frac{C_u}{f_u}$$

$$C_b = \left[\frac{1-H}{f_u} + H \times \rho\right] C_u$$

In the above equation, C represents the total plasma concentration, C_u represents unbound concentration, C_b represents total blood concentration, f_u is the unbound fraction, ρ is the erythrocyte to plasma concentration ratio, and H shows the hematocrit levels. Here, we can understand that the total blood concentration is directly proportional to the unbound plasma concentration when the unbound plasma fraction is constant (Londhe and Rajadhyaksha, 2020).

When drugs have low affinity to plasma proteins, the unbound fraction f_u is unity. The total drug concentration in the plasma also depends on various factors such as the age of the patient, nature of the disease, condition of the patient, burns or not etc. additionally, it also varies largely between species (Rowland and Emmons, 2010).

Blood to plasma ratio is the ratio defining the concentration of drug in the plasma to the concentration of drug in the plasma and is represented as "*R*" (Saha *et al*., 2017). Other the aforementioned physical precondition of the body, another factor that influence the total plasma concentration involves the nature of the drug.

Drugs which are acidic in nature tend to be hydrophilic. These types of drugs include Doxycycline, Valproic acid, Clindamycin, Vincristine, Chlorthalidone, Ibuprofen to name a few. These drugs apart from being acidic in nature exhibit higher affinity to plasma proteins and very little toward the erythrocytes. These drugs show erythrocyte to plasma concentration ratio $\rho < 1$ approximately in the range of 0.55 to 0.60.

Similarly, drugs which are basic exhibit higher affinity towards erythrocyte and the amount of plasma bound proteins are less. Under such conditions, the total drug concentration is very little affected by the changes in the protein binding as majority of the drug is membrane bound.

Due to such large anomalies existing in a human body under the influence of drugs, consideration of blood to plasma concentration should be analyzed and considered while studying as well as optimizing a suitable DBS/DPS method (Rowland and Emmons., 2010).

Matrix choice

In case, the calibration curve generated during the study was prepared in blood mixed with anti-coagulant, it is necessary to conduct studies in similar matrix. When blood sample is procured from patients, it becomes necessary for the patients to provide blood mixed with anti-coagulant. This is achieved by preconditioning the DBS/DPS card with anti-coagulant. If studies have to be carried out on DBS/DPS directly (in the absence of anti-coagulant) then the calibration curve has to be prepared in blood without anticoagulant. This is not possible as studies on blood without anti-coagulant will instantly start clotting and won't be possible to proceed further. In such circumstance, we have to consider the role of anticoagulant in the study; however, due to the limited knowledge about the interaction of drugs and anticoagulant, it becomes difficult to select correct anticoagulant and determine its volume (Capiau *et al*., 2019).

Storage and sample handling

DBS/DPS method involves handling small volume of blood or plasma depending on the type of study. Various factors like humidity, temperature, storage condition/refrigeration condition, and storage device can show significant variation in terms of study results. Optimization of theses parameters help increase the precision and accuracy in these studies (Capiau *et al*., 2019).

Matrix ageing and storage temperature are major concern when developing DBS/DPS method. Different parameters such as precision and accuracy, recovery, solution stability, drug degradation, and selectivity can vary based on the age of the matrix. Improper handling of samples can lead to oxidative degradation of drug. These are mostly is seen in aged matrix as due to degradation of drug; the recovery and selectivity can vary thereby altering these parameters drastically.

In order to reduce the impact of ageing of matrix in the detection of drugs, optimization of stability parameters such as temperature as well as long-term matrix stability have to be performed to ensure similar recovery every time the protocol is followed. Proper handling of the sample has to be ensured, especially the package and temperature restrictions have to be strictly enforced to receive the matrix which is in good shape and provides good analysis for the study (Londhe and Rajadhyaksha, 2020).

Sample preparation

One of the major roadblocks in the development of a protocol for DBS/DPS is low volume of the sample. Conventionally, sample preparation can be optimized based on the prevalent most preferred techniques such as SPE, LLE, or Protein Precipitation. However, Protein Precipitation is preferred as it is a one step process with minimal loss of sample.

Recently extraction of the sample using automated systems is gaining popularity since 2009, where implementation of robotic arm replaces conventional manual extraction processes (Demirev *et al*., 2013). This not only reduces manual labor but also reduces sample loss due to improper equipment handling.

Apart from selection of extraction procedure, it is important to understand whether the study will be conducted based on the spot volume or punch diameter. If study is conducted based on spot volume, then the necessary optimization as mentioned above has to be done. If not, then the punch location plays important role in the recovery of the drug from the spot. In a study conducted by Moat *et al*. (2020), they suggested that smaller volume resulted in spreading of the blood and therefore reducing the concentration of in the sub punch. On the contrary, when larger volume of blood (50–75 µl) is used, then it was observed that larger volume of blood was obtained in the sub punch yielding increased recovery.

Another study performed by Hall *et al*. (2015) suggested that the DBS diameter shows a logarithmic relationship to the total spot blood volume suggesting that the per punch blood volume increases exponentially as the diameter of the sub punch is increased (Hall *et al*., 2015; Moat *et al*., 2020).

A study reported by O'Mara *et al*. (2011) suggested that the location of sub punch could drastically change the accuracy of the study. Hence suggested replicating the spot location if samples have to be resampled to incur a bias ±15%. They suggested that that certain type of filter paper or with variation in the haematocrit test (HCT) level showed a phenomenon called "volcano effect" where the concentration of the drug in the peripheral is more than that in the center (O'Mara *et al.,* 2011). Another study performed by Moat *et al.* (2020) also reported a similar volcano effect when they studied relation between sub punch location to recovery (Fig. 4).

Hematocrit effect

It is been known that the increase in the erythrocyte concentration increases the viscosity of the blood. This effect is referred to as "Hematocrit effect." Due to variation in the viscosity of the blood, hematocrit effect is seen to negatively impact the precision and accuracy as well as spot area. This often leads to production of an unclear sample which can lead to unwarranted matrix effect. HCT values vary with age, precondition of the person such as anemia, severe burn, gender, living conditions, and nutrition quality. De Kesel *et al.* (2013) suggested that the HCT values are the highest in neonates secondly men followed by women. Lim in his review suggested that the spread of blood on any filter paper is largely influenced by the HCT level of the blood.

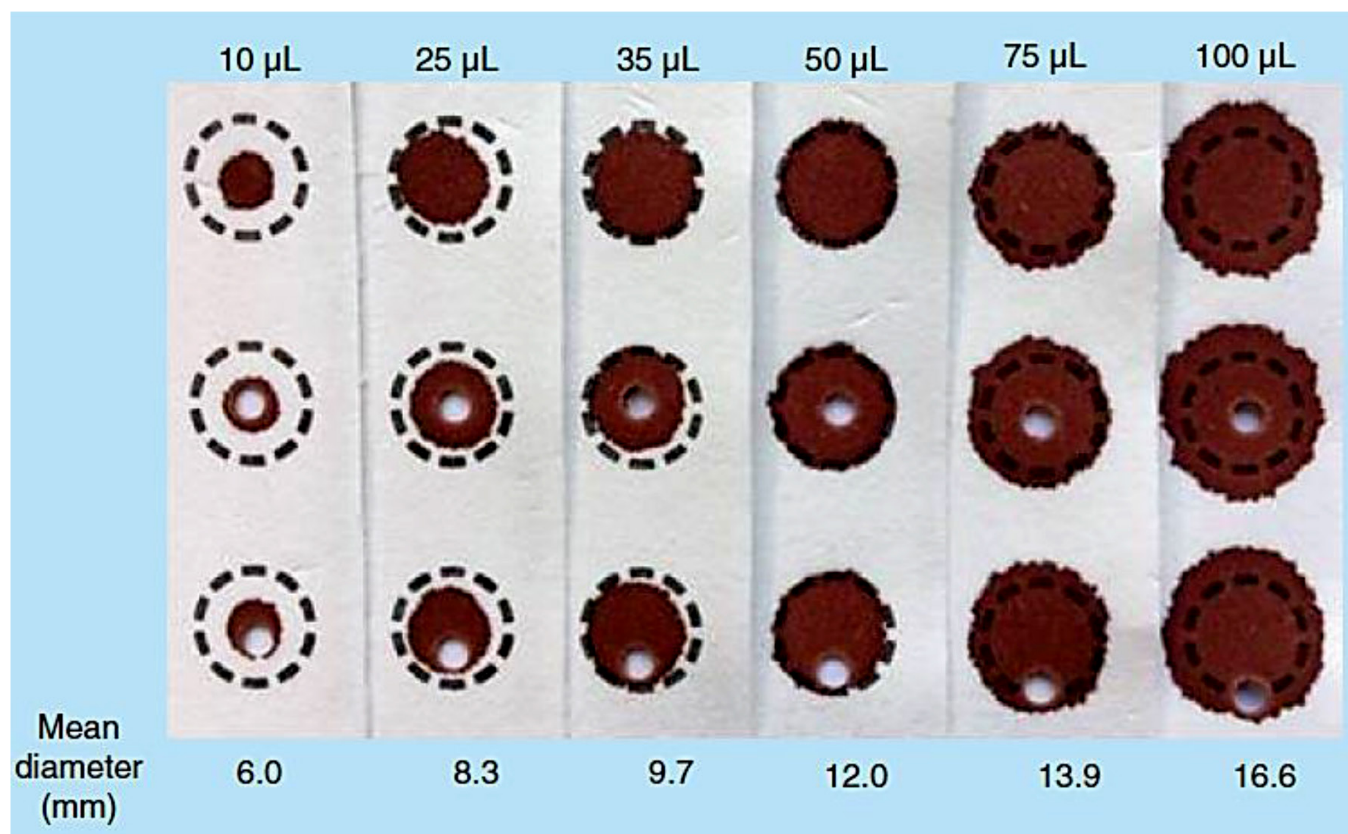

Figure 4. Punch location studies performed by Moat *et al.* (2020).

A paper published by De Kesel et al. (2013) deeply discusses the implications of this parameter in the DBS studies.

The following as some of the impacts are related to HCT levels of blood:

Blood comprising of a higher HCT level seems to spread less, and therefore forms a small spot. Therefore, larger volumes of blood will be extracted from sub punches obtained from higher HCT sample as compared to the lower HCT sample when the diameter of the sub punches are maintained constant. This not only affects the robustness of the method but also reduces the reproducibility of the result. HCT is also an important parameter which is related to the blood to plasma ratio as mentioned above. When the HCT values increases, the blood to plasma ratio decreases considering the plasma concentration to be constant and while ignoring the effect of *HCT on the spread of DBS*

The HCT effect is greatly ruled by the nature of the drug. Drug which are highly attracted to the erythrocytes show more affinity toward RBCs. Therefore, under such circumstances, the HCT value becomes an influencing parameter. In order to reduce the influence of the varying HCT concentration, volumetric blood spot is required which is difficult to obtain without skillful and trained personnel especially at home without guidance. Selection of the matrix based on the property table as suggested by Rowland and Emmons (2010). Alternatively use of dried plasma could also help in alleviating the problem. However, using non cellulose based substrate or glass paper filters, alginate and chitosan foams are some of the suggested alternatives according to review report presented by Rowland and Emmons (2010) (Fig. 5).

Recent Advances of DBS and DPS

The last few years, lots of development is seen in the aspect of drug detection from DBS/DPS coupled with LC-MS/MS. The predominant method of extraction seen in these papers were protein precipitation. The modification seen with respect to the method of extraction were either evaporation post precipitation followed by reconstitution or Protein Precipitation followed by LLE.

However, use of SPE and automation has also been reported by a few. The validation of these methods has been mostly done in accordance with FDA and industrial guidelines as well as few important criteria such as hematocrit values, spot volume have also been studied as mentioned above. The recent advances in the field of DBS and DPS (combined with LC-MS/MS) in the last four years are shown in the Tables 1 and 2 respectively.

Scope of DBS/DPS

The first report of using DBS for TDM was in 1978 by Albani and Toseland (1978) for the detection of theophylline in gas chromatography. The number of assays with regards to the detection of drugs using DBS/DPS has been increasing since. Prospective groups on whom DBS/DPS is most applicable includes

Neonatal patients/infants and pediatric group

Minimal invasive technique is required when the patient is a neonate or an infant. Since conventional method of drug detection involves extraction of large amounts of blood approximately 2ml of blood per plasma-based assay, it is only convenient to extract samples via DBS/DPS. Due to ease of sample handling the loss of sample due to inefficient handling can be minimized.

Expectant mothers

Pregnant women who have risk of epilepsy, are treated with doses of antiepileptic drugs such as lamotrigine and oxcarbazepine. Close monitoring of such drugs has to be performed

Blood:plasma ratio	Hematocrit constant†	fu constant	ρ constant	Plasma and/or DBS
0.55 < 2.0	Y	Y	Y	Plasma or DBS can equally be used
0.55 < 2.0	Y	N	Y	Plasma or DBS can be used, but the reasons for variability in plasma protein binding need to be understood and accommodated
≥2.0	Y	Y	Y	DBS is preferred due to hemolysis concerns
≥2.0	Y	N	Y	DBS is preferred due to hemolysis concerns
≥2.0	Y	Y	N	Depending on the situation and the objectives of the study either plasma or DBS can be used, but if DBS is used the reasons for the lack of constancy in the blood cell partitioning need to be understood and accommodated
Any	N	Y/N	Y/N	Decision depends on value of R as in above scenarios with the additional need to correct for hematocrit when using DBS if R approaches 0.55 or ≥2.0

†By constant it is meant that the parameter value varies to a sufficiently small extent not to materially affect the relationship between unbound plasma and total concentrations.
DBS: Dried blood spot; fu: Unbound fraction in plasma; N: No; R: Blood-to-plasma concentration ratio; Y: Yes.

Figure 5. Guide to decide the matrix- DPS or DBS (Rowland *et al*., 2010).

Table 1. Recent advances in DBS in combination with LC-MS/MS.

Drug	Filter paper	DBS/DPS	Method of extraction	Reference
Risperidone, Aripiprazole, Pipamperone Major active metabolites: 9-OH-risperidone, Dehydroaripiprazole	Whatman 903	DBS	Ultrasonic assisted PPT	Tron *et al*., 2017
Propafenone, 5-hydroxypropafenone, N-depropylpropafenone	Whatman 903	DBS-EDTA anticoagulated	PPT	Andy *et al*., 2017
25-hydroxyvitamin D	Whatman 903	DBS-venous blood (Hct-0.39)	PPT followed by LLE	Jensen *et al*., 2018
Amodiaquine, Chloroquine, Quinine, Sulfadoxine, Pyrimethamine, Mefloquine, Lumefantrine. 2 active metabolites: N-desethyl-amodiaquine, Desbutyl-lumefantrine	Whatman 903	Adult DBS and neonatal DBS.	PPT with 1% formic acid and methanol	Gallay *et al*., 2018
Alprazolam, Amphetamine, Cocaine, Codeine, Diazepam, Fentanyl, Lysergic acid diethylamide, 3,4-methylenedioxymethamphet-amine, Methadone, Methamphetamine, Morphine, Oxycodone.	FTA-DMPK-B	DBS	Automated	Gaugler *et al*., 2018
Benznidazole	Ahlstrom TFN filter paper	DBS	LLE	Galindo Bedor *et al*., 2018
Voriconazole	Whatmann 903	DBS	PPT	Martial *et al*., 2018
Docetaxel	FTA-DMPK-C	DBS	PPT	Raymundo *et al*., 2018
Tenofovir, Emtricitabine, Lamivudine	Whatman 903	DBS	PPT followed evaporation and RS	Schauer *et al*., 2018
Dexmedetomidine	Whatmann 903	DBS	LLE	Rivera-Espinosa *et al*., 2019
Chloroquine	Whatmann 903	DBS	SPE	Kaewkhao *et al*., 2019
Fentanyl analog	FTA-DMPK A, B or C	DBS	LLE	Seymour *et al*., 2019
Ivermectin	Whatman 903	DBS	PPT	Duthaler *et al*., 2019
Vancomycin, Creatinine, Teicoplanin	Whatman 903	DBS	LLE	Scribel *et al*., 2019
Trimethylamine N-oxide, Trimethylamine, L-carnitine, Choline, Betaine, γ-butyrobetaine, Nε ,Nε 47 ,Nε-trimethyllysine, 48 dimethylglycine, Homocysteine	Whatman 903	DBS	PPT	Cho *et al*., 2019
Benzodiazepines, NPS- Novel Psychoactive Substances, Neuroleptics, Opioids, Antidepressants, Synthetic cannabinoids, Amphetamines, Anticonvulsant, Cocaine, Cannabinoid	Whatman 903, FTA-DMPK-A, FTA-DMPK-B, FTA-DMPK-C	DBS	PPT followed by LLE	Joye *et al*., 2019
Carbamazepine, Valproic acid, Phenobarbital, Phenytoin one active metabolite: Carbamazepine-10,11-epoxide	PerkinElmer 226 Bioanalysis RUO cards	DBS-venous blood (Hct-0.42)	Automated DBS-MS 500 system CAMAG	Velghe *et al*., 2019
Methadone Metabolites: 2-ethylidene-1,5-dimethyl-3,3 diphenylpyrrolidine and 2-ethyl-5-methyl-3,3-diphenylpyraline	Whatman 903	DBS	Salt Assisted PPT	Davari *et al*., 2020
Ceftolozane	Whatman 903	DBS	LLE	Martens-Lobenhoffer *et al*., 2020
Ganciclovir	FTA- DMPK-C, Whatmann 903 and 3 MM Chr.	DBS	Ultrasonic Assisted PPT	Rower *et al*., 2020
Vancomycin, Creatinine And Teicoplanin	Whatman 903	DBS	LLE	Scribel *et al*., 2019

Table 2. Recent advances in DPS in combination with LC-MS/MS.

Drug name	Filter paper	Matrix used	Extraction technique	Reference
Busulfan	Whatman 903	DPS	PPT-automated	Dilo *et al.*, 2020
Warfafrin	Whatman 903	DPS rat plasma	PPT	Chernonosov, 2016
Atenolol	Whatman 903	DPS rat plasma	PPT	Chernonosov and Koval, 2019
Abiraterone Acetate	FTA-DMPK-C	DPS	PPT	Bhatnagar *et al.*, 2019
Ritonavir	DMPK C cards	DPS dog plasma	PPT followed evaporation and RS	Li *et al.*, 2015
Amikacin, Kanamycin B, Creatinine.	Whatman 903 Chromafil-Xtra PTFE-20/13 sample filters (pore size of 0.22 m).	DPS	PPT	da Silva *et al.*, 2020
Haloperidol, Aripiprazole, Olanzapine, Quetiapine, Clozapine, Risperidone, And Paliperidone	FTA DMPK-A	DPS	PPT	Ruggiero *et al.*, 2020
Levetiracetam, Lacosamide, Topiramate, Ethosuximide, Lamotrigine, Rufinamide, Zonisamide, Primidone, And Oxcarbazepine Active Metabolite 10-OH-Monohydroxycarbazepine		DPS	PPT	D'Urso *et al.*, 2019
Morphine, Codeine, Oxycodone, Hydrocodone, Amphetamine, Methamphetamine, 3,4-Methylenedioxymethamphetamine, Phentermine, And Mephedrone	RBC filter- iPOCDX membrane filter sheet. Bottom support disks, card stock material	DPS	PPT-automated	Ryona and Henion, 2016

as altered serum levels in patients can alter the metabolism of such drugs (Wegner *et al*., 2010). In order to profile such drugs, spot blood method can be implemented thereby drastically reducing the amount of blood required for its detection.

Elderly people

Certain old age-related problems such as dementia and Alzheimer's cannot independently access phlebotomist. Therefore, such simple method of blood sample collection could help out the care givers to collect blood samples for drug detection. People suffering from Parkinson's disease who suffer from hand tremors and are unable to keep hand still in order to collect blood samples, as well as arthritic patient who face great difficulty in moving hand joints, could benefit from DBS/DPS.

Drug influenced anemic patient

Certain drugs cause anemia in patients. It is studied that certain medical condition also cause anemia; one such example is malarial anemia (White, 2018). Therapeutic drug monitoring in such patients is difficult as extracting large amounts of blood from these patients could adversely affect the health of the patients. DBS/DPS could be employed in such cases.

Diabetic patient

Diabetic patients visit healthcare facilities with medical conditions such as increased blood glucose levels, high blood pressure, and alteration in electrolyte level in their blood. DBS/DPS could help researchers to collect minimal amount of blood for TDM of drugs in such patients. This will not only improve their quality of life but also greatly reduce their frequency to health care facilities. One such drug—sitagliptin has been developed focusing on treating diabetes. TDM of such drugs require patient samples where DBS has been employed (Scherf-Clavel and Högger, 2015).

Pros and Cons of DBS/BPS

Pros of DBS/DPS method is

1. Unlike any other method, DBS has both advantage and disadvantages (Velghe *et al*., 2018; Wilhelm *et al*., 2014). The pros of DBS are:
2. Ease of sample collection. This procedure is not and extensively invasive method. Patients are required to prick and spot the blood.
3. Non time consuming: Patients are not required to visit sample collection center and spend time in blood collection. This process can be done at home without the assistance of a phlebotomist.

4. Ease of transportation: Blood harvested by patient's samples are stored in vial and has to be cautiously carried to avoid spillage and contamination. Sterility has to be maintained to prevent bacterial and viral contamination. Careful monitoring of temperature has to be done. DBS surpasses all these limitation by allowing the blood to dry and can be stored in plastic pouches with no fear of cross contamination or bacterial contamination,
5. Less volume is required. For monitoring of drug through conventional method requires large amounts of blood sample. With the advent of micro-sampling method, it is possible to extract drug.
6. Clear sample produced after extraction from DPS. DBS however needs optimization to produce a clean sample.

Cons of DBS/DPS method is

1. Sensitive detection technique is requited as the volume of sample extracted is very small.
2. New sample/fresh sample is required to study each different parameter. This requires the patient to prepare the DBS/DPS every time new parameter have to be studied.
3. During clinicals study of any lead drug candidate, it is required to collect sample in regular intervals. Collection of DBS/DPS under such circumstance will require patients to undergo multiple pricking session which is inconvenient.
4. It is important to study the correlation of drug concentration between the venous blood and the capillary blood.
5. If any study is performed wrongly or does not seem to match the validation criteria, there will not be any spare sample for repeat of the experiment.
6. One of the major concerns regarding the DBS method is the impact of hematocrit values (HCT values) on the spotting size, the homogeneity and as well as will impact the study method.
7. Possibility of contamination of the sample during transportation if the pack is damaged during handling.

DISCUSSION

DBS/DPS can prove to be a cost-effective sampling technique which not only encourages patients to reduce their frequency to the hospitals but also efficiently increases the quality of life. A study conducted by Martial *et al.* (2016, 2018) in Netherlands which is a part of the Dutch Government Funded project evaluated the variance in cost in both conventional as well as self-sampling settings.

They reported that the DBS/DPS significantly lowers the travel costs of the patients and the cost expenditure caused due to loss of productivity due to time spent at hospital, time required to sample, travel cost, and sample sending is less

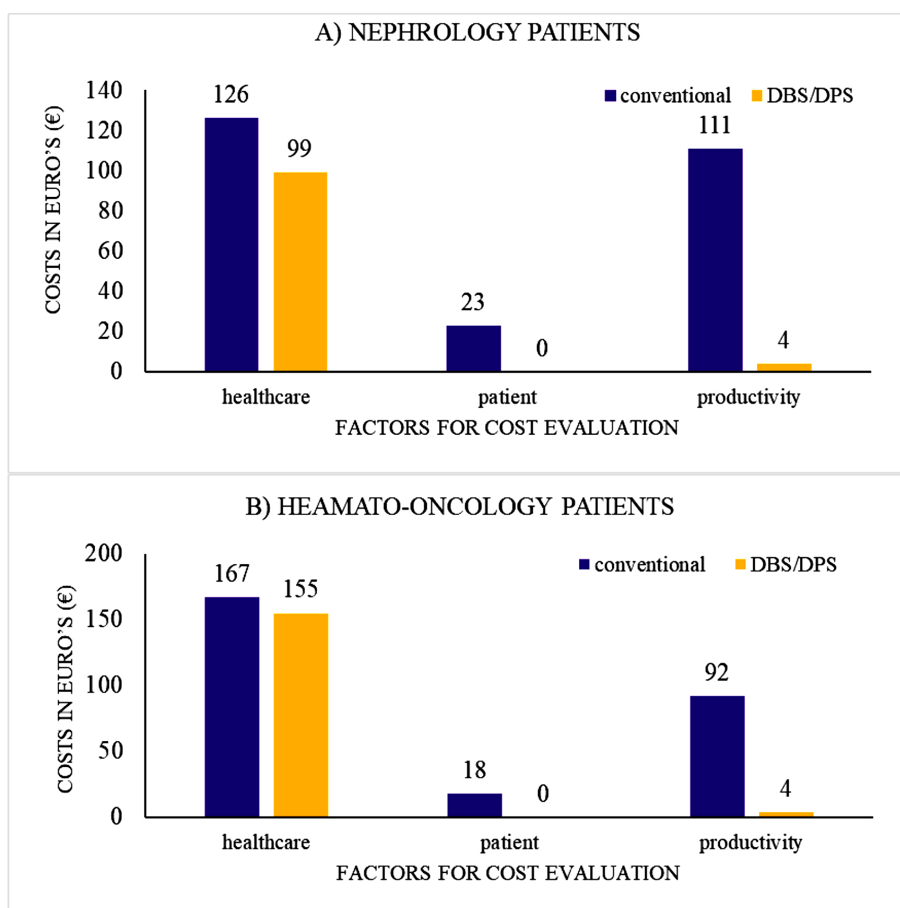

Figure 6. (A) Cost evaluation of conventional sampling as well as self-sampling as explained by Martial *et al.* (2016) of nephrology patients. (B) Cost evaluation of conventional sampling as well as self-sampling as explained by Martial *et al.* (2016) of hemato-oncology patients.

than 95%. They also suggested that the cost for sampling in a conventional sampling setup is significantly lowered approximately 2.5 times when in case of nephrology patients and 1.8 times in case of haemato-oncology patient when performed as dried spots. A graph chart evaluating various costs involved in both conventional sampling and self-sampling set up for nephrology patients (A) and haemato-oncology patient (B) is represented in Figure 6A and B as explained by data reported by Martial *et al.* (2016).

CONCLUSION

Since the introduction of DBS/DPS in the 1960s, this sampling method has gained fame for its low volume and non-invasive technique. Several developments have been made using DBS/DPS as a sampling method coupled with other detection devices such as UV has been widely implemented such as for detection of anti-epileptic drugs- evetiracetam, lamotrigine, ethosuximide, felbamate, rufinamide, zonisamide, and monohydroxy-carbamazepine by Baldelli *et al.* (2015) and detection of a non-nucleoside reverse transcriptase inhibitor - Efavirenz by Hoffman *et al*. (2013).

The advent in terms of spot drug detection for therapeutic drug monitoring using LC-MS/MS has been gaining popularity owing to its extremely sensitive and selective method of detection. The requirement of small sample volumes and precise detection of the molecules has makes LC-MS/MS detection as a preferred choice when it comes to TDM-Therapeutic Drug Detection.

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CONFLICT OF INTEREST

There is no potential conflict of interest with this article.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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