Analytical and Bioanalytical Chemistry (2022) 414:7085–7101
https://doi.org/10.1007/s00216-022-04221-1

REVIEW

Dried blood spots in clinical lipidomics: optimization and recent findings

Helena Beatriz Ferreira[1,2] · Inês M. S. Guerra[2,3] · Tânia Melo[1,2] · Hugo Rocha[4,5] · Ana S. P. Moreira[1,3] · Artur Paiva[6,7,8] · M. Rosário Domingues[1,2]

Received: 5 April 2022 / Revised: 15 June 2022 / Accepted: 5 July 2022 / Published online: 15 July 2022

Abstract

Dried blood spots (DBS) are being considered as an alternative sampling method of blood collection that can be used in combination with lipidomic and other omic analysis. DBS are successfully used in the clinical context to collect samples for newborn screening for the measurement of specific fatty acid derivatives, such as acylcarnitines, and lipids from whole blood for diagnostic purposes. However, DBS are scarcely used for lipidomic analysis and investigations. Lipidomic studies using DBS are starting to emerge as a powerful method for sampling and storage in clinical lipidomic analysis, but the major research work is being done in the pre- and analytical steps and procedures, and few in clinical applications. This review presents a description of the impact factors and variables that can affect DBS lipidomic analysis, such as the type of DBS card, haematocrit, homogeneity of the blood drop, matrix/chromatographic effects, and the chemical and physical properties of the analyte. Additionally, a brief overview of lipidomic studies using DBS to unveil their application in clinical scenarios is also presented, considering the studies of method development and validation and, to a less extent, for clinical diagnosis using clinical lipidomics. DBS combined with lipidomic approaches proved to be as effective as whole blood samples, achieving high levels of sensitivity and specificity during MS and MS/MS analysis, which could be a useful tool for biomarker identification. Lipidomic profiling using MS/MS platforms enables significant insights into physiological changes, which could be useful in precision medicine.

Keywords Dried blood spots · Mass spectrometry · Lipidomics · Disease biomarkers

Abbreviations

AA	Arachidonic acid
ACN	Acetonitrile
BHT	Butylated hydroxytoluene
CE	Cholesteryl esters
Chol	Cholesterol

✉ Helena Beatriz Ferreira
helenabeatrizferreira@ua.pt

✉ M. Rosário Domingues
mrd@ua.pt

[1] Mass Spectrometry Center & LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

[2] CESAM–Centre for Environmental and Marine Studies, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

[3] CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

[4] Newborn Screening, Metabolism and Genetics Unit, Human Genetics Department, National Institute of Heath Doutor Ricardo Jorge, 4000-053 Porto, Portugal

[5] Superior School of Heath, Polytechnic Institute of Porto, 4200-072 Porto, Portugal

[6] Unidade de Gestão Operacional Em Citometria, Centro Hospitalar e Universitário de Coimbra (CHUC), 3004-561 Coimbra, Portugal

[7] Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, 3000-370 Coimbra, Portugal

[8] Instituto Politécnico de Coimbra, ESTESC-Coimbra Health School, Ciências Biomédicas Laboratoriais, 3046-854 Coimbra, Portugal

CH_2Cl_2	Dichloromethane
DBS	Dried blood spots
DHA	Docosahexaenoic acid
DI-MS	Direct injection mass spectrometry
EPA	Eicosapentaenoic acid
FA	Fatty acids
GC-FID	Gas chromatography with flame ionization detection
GC-MS	Gas chromatography coupled with mass spectrometry
Hct	Haematocrit
HDL	High density lipoproteins
HPLC	High-performance liquid chromatography
IPA	Isopropyl alcohol
LC-MS	Liquid chromatography coupled with mass spectrometry
LDL	Low density lipoprotein
MeOH	Methanol
MS	Mass spectrometry
MTBE	Methyl *tert*-butyl ether
MS/MS	Tandem mass spectrometry
P	Plasmenyl
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PL	Phospholipids
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acids
RP	Reverse phase
SFC-MS	Supercritical fluids chromatography coupled with mass spectrometry
SM	Sphingomyelins
SPME	Solid-phase microextraction
TG	Triacylglycerols
TLC-GC-FID	Thin layer chromatography followed by gas chromatography with flame ionization detection

Introduction

Dried blood spots (DBS) are a less invasive method for blood collection and are being considered as an alternative to whole blood samples obtained by venipuncture or arterial sampling. DBS are widely used in clinical environment to collect samples for newborn screening, as a part of public health policies [1]. This method is also considered promising for clinical diagnostics and for precision medicine, since it has been reported as an effective method for pre-analytic stages of diagnostics [2, 3]. Research on DBS for application in medicine is growing and is being explored in combination with mass spectrometry (MS) [1] and in the context of different omics, like metabolomics [4, 5], proteomics [6], and more recently lipidomics. While DBS is already well established in metabolomic analysis, its use in proteomics seems to face some challenges regarding protein analysis and degradation. Lipidomics is starting to be associated with DBS, once lipids are reported to be stable enough to endure the timespan between sample collection and lipid extraction, even more stable than in traditional samples (plasma/serum/whole blood). The major research work on this method has been done concerning pre-analytical and analytical steps and procedures, and very few in application to real clinical investigations.

Despite being a field scarcely studied, lipidomic analysis with DBS shows promising results, revealing the need for more work to be done. In this review, we will address the most important findings from the use of DBS in lipidomics, highlighting as well future perspectives and development needed to pinpoint DBS as a good sampling and storage method, prior to lipid analysis for biomarker discovery, and application in clinics, diagnostics, and/or therapeutic drug monitoring.

Advantages and applications of DBS to study lipids in biological samples

The DBS is a microsampling method that is very simple and easy to perform, as it requires only the depositing of small volumes (μL) of capillary blood onto DBS cards [1]. The collection of blood is easier; therefore, the sample can be collected at home, and it does not demand specialized entities to do so. The patient can perform the sample collection by its own (or the parents, in the case of small children). The blood is placed directly onto the DBS card until the drawn circle area is full. Yet, the skin must not be punctured with the same lancet more than once due to risk of bacterial contamination and infection [7]. Contrarily to venipuncture and arterial sampling, DBS samples do not need to be immediately stored in refrigerated conditions after its collection. This way, the transport and accommodation of these type of samples is very straightforward which allows the creation of simplified biobanking facilities for storage [8]. Venipuncture and arterial blood collection are the most common blood collection methods; however, they are not considered to be the best way to collect a blood sample [9]. These methods have to be performed exclusively by personnel who have received proper training. Many patients find it inconvenient (due to dietary restrictions) and disturbing. Also, there are also risks related with the puncture site cleansing, storage, transportation, and potential loss or contamination of the blood samples once they are collected [9]. Thus, DBS have several advantages when compared with arterial sampling and venipuncture, as described in Fig. 1.

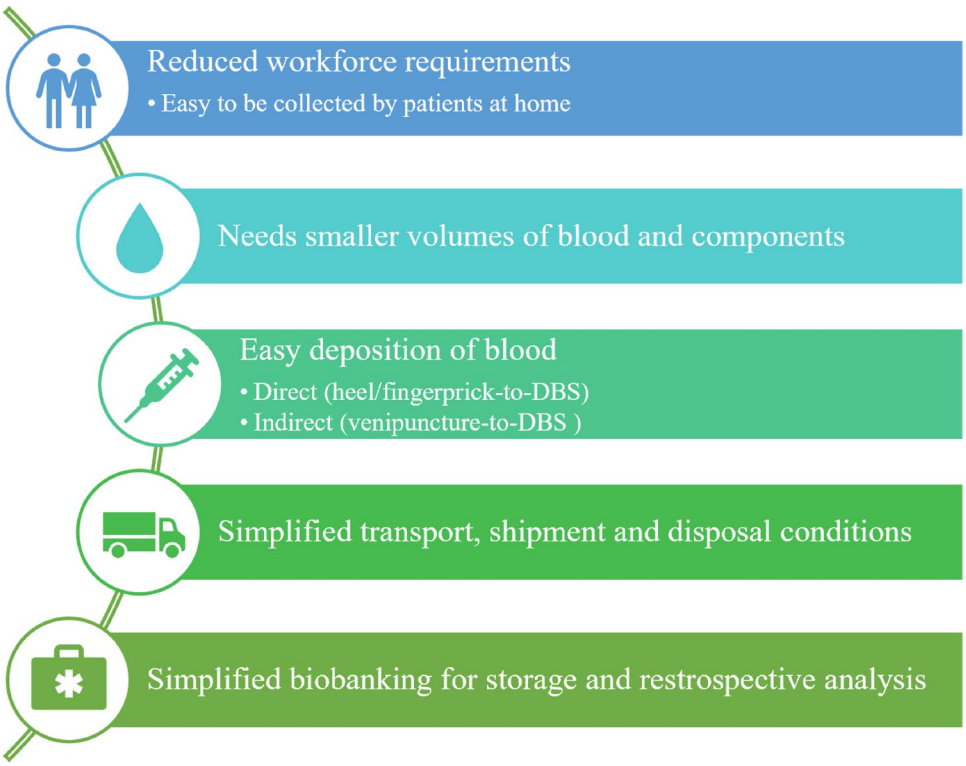

Fig. 1 Dried blood spots (DBS) sampling method offers several advantages comparing with arterial/venipuncture samples. It is a minimally invasive sampling process, which can be performed by the patient on their own, and it is convenient to store and transfer allowing long distance shipments

DBS samples enable longitudinal studies without sample degradation if they are stored under the right conditions according with the analyte of interest. Due to high stability of several blood components, like lipids, it is possible the detection of a high number of analytes in a single analysis. The physician has a broader understanding of the patient's metabolism and can reach a more fitting diagnosis [10].

DBS have three primary applications (Fig. 2): in health-care services (in a clinical scenario for screening/diagnostics/follow-up), in research and surveillance studies into undeveloped populations, and in drug development/monitoring [10, 11].

Lipids are important mediators in pathological conditions; therefore, its analysis could detect the early development of some diseases or even be considered as biomarkers of disease status, and be useful to evaluate the therapeutic outcomes [12]. Lipids are promising in the search of new putative biomarkers for diagnosis and evaluation of disease progression or to unravel the role of lipids in the disease pathophysiology. As pointed out before, all the advantages that DBS offers combined with the fact that lipids are quite stable in DBS [13] make DBS suitable for its use in lipidomic analysis. It is a promising field of research, with potentially favourable applications in clinical environments. However, the association of DBS with lipid/lipidomic analysis is still in its infancy, mostly because lipidomics is an omics field still in the early stage of development but with high potential applications [13], and there is not much information regarding its use in this area. DBS method is well-established in other omics, especially metabolomics [14], and there is a need for more investigation on its applicability on the lipidomics field.

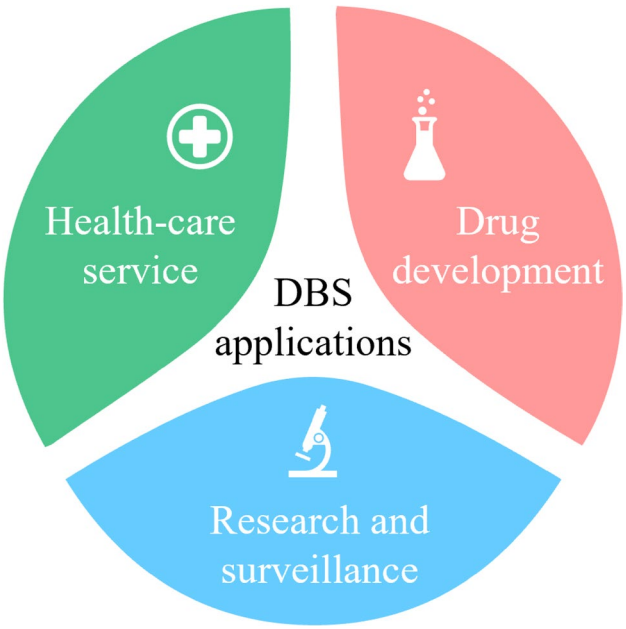

Fig. 2 Dried blood spots (DBS) main applications: health-care services (in a clinical scenario for screening/diagnostics/follow-up), research and surveillance studies, and drug development/monitoring

In this review, we aimed to gather lipidomic investigations using DBS cards. For that, English language publications were identified through a computerized search (PubMed database) until June 2021 using the following keywords: lipidomic(s), lipid(s), lipid profile(s), phospholipid(s), fatty acid(s), and sphingomyelin(s) combined with dried blood spot(s). A total of 26 publications were found. From those, a total of 21 papers were selected according with the eligibility criteria chosen. We only considered research studies that used gas chromatography coupled with mass spectrometry (GC–MS), gas chromatography-flame ionization detection (GC-FID), liquid chromatography coupled with mass spectrometry (LC–MS), direct injection mass spectrometry (DI-MS), and supercritical fluid chromatography coupled with mass spectrometry (SFC-MS). Studies that did not report the use of those techniques, or were review papers, were not taken into consideration. From the 21 eligible studies, most of them focused on methodology/protocol optimization, from pre-analytical parameters evaluation to analysis optimization, and very few in actual clinical lipidomic investigations, as will be described.

Most of the papers on the evaluation of pre-analytical variables that can affect the quality of the results of lipid analysis were focused on (i) the extraction efficiency of different methods, (ii) factors that may affect the recovery rate of the lipids, like the type of DBS card, storage with antioxidants, and matrix effects, and (iii) lipid stability over a period of time. Others studied the DBS method validation in clinical cases as a possible candidate to future clinical applications by comparing the results with traditional methods (plasma, serum, or whole blood) [13, 15–29].

Pre-analytical parameters that may affect lipid extraction from DBS

The workflow of DBS starts with blood collection in the paper card, followed by storage/transport, lipid extraction, and analysis. After blood collection, the analyte extraction procedure starts by punching a circle (with a set diameter) from the DBS card. Then, the analyte is extracted with selected extraction solvents and protocols that must be carefully chosen according with the physico-chemical properties of the analyte. Nonetheless, different pre-analytical parameters may affect the amount of compound and the lipid extract recovered from DBS. The parameters include the transport and storage conditions, the type and quality of the paper of the DBS card (variations in the matrix affects the blood volume, the extraction efficiency, and the chromatographic results), and haematocrit (Hct). These are probably the most impactful factors to significantly affect the results; this way, the procedures must be optimized and standardized to increase the reliability and reproducibility of the assay and allow the comparison of research work data between different labs, promoting harmonization of lipidomic investigations.

Storage of DBS and stability of lipids

The solid nature of DBS paper cards and adsorption capacity make analytes less reactive (thus more stable) than in whole blood, plasma, or serum samples thus having higher stability at room temperature, at least for a week [30]. It is also an advantage to monitor metabolic diseases diagnosed in newborn screening programs and could be promising for sample collection for other diseases that need monitoring. To maintain analyte stability and to control storage conditions, to make the sample stable during longer times, transport to the laboratory should be made as soon as possible. Storage conditions must account for several variables such as temperature, humidity and time within field, transport, and laboratory settings [31]. Temperature and humidity conditions have a direct impact on the stability of several analytes including lipids, amino acids, and DNA [32–34]. Temperature effect can be prevented by keeping samples in refrigerated/cooled conditions. However, reducing the storage temperature is not always the solution for all analytes as it is described for polyunsaturated fatty acids (PUFA). It was reported that few PUFA (20:4, 20:5, and 22:6) suffer significant degradation after 10 days of storage at − 28 °C [33]. Humidity, which can also cause lipid degradation by hydrolysis reactions, can be avoided by confining DBS cards in sealed bags with desiccants while transportation or storage [1]. Nonetheless, optimal storage settings must be determined for the analyte in question and the purpose of the study (momentary or longitudinal). In the case of lipids stability in DBS, studies reported that PUFA are stable between 21 days and 2 months at room temperature [21, 23] and, in the case of phospholipids (PL), up to 2 weeks at 4 °C or room temperature [13]. Nonetheless, there is a need for more studies on the stability of molecular species since most of the studies were performed on FA.

Type of DBS cards

There are plenty of DBS cards available on the market, and different studies used DBS cards from different manufacturers (Table 1). The difference on the card type between studies may explain the variance of results due to matrix effects. Each manufacturer produces the DBS cards with a specific chemical composition, fibre density, and network. This way, the same sample could present dissimilar results depending on the DBS card type and, consequently, on the matrix effect. Liu et al. intended to develop a lipidomic DBS method that would allow samples to be stored at room temperature for at least 2 months [23]. For that, the team

Table 1 Analysis of the selected studies according with the type of DBS card, punch diameter, use of stabilizers, and lipidomic approach

DBS card type	Diameter of the punched disk	Stabilizers	Lipidomic approach	Reference
Guthrie cards				
Commercially available Guthrie spot cards	3.2 mm	Chaeotropic buffer	DI-MS	Furse et al. (2020) [16]
Whatman 903 ™				
Whatman 903™ protein saver cards	3.0 mm	-	LC–MS	Primassin et al. (2010) [17]
Whatman chromatography paper	100 mm^2	BHT	GC–MS	Metherel et al. (2013) [21]
Whatman 903® specimen collection paper	3.2 mm	-	LC–MS	Chuang et al. (2014) [35]
Whatman 903™ CF12® protein saver cards	144 mm^2	-	LC–MS	Luginbühl et al. (2016) [22]
Whatman 903™ filter paper cards with BHT antioxidant	144 mm^2	BHT	GC–MS	Mashavave et al. (2016) [36]
Whatman 903™ protein saver cards	144 mm^2	-	GC–MS	Drzymała-Czyz et al. (2017) [25]
Whatman 903™ filter paper cards	6.0 mm	LiCl	DI-MS	Gao et al. (2017) [13]
Whatman 903™ protein saver cards	3.0 mm	-	LC–MS	Liao et al. (2018) [27]
Whatman 903™ filter paper cards	160 mm^2	Different antioxidants (pure/mixture)	GC-FID	Marino et al. (2018) [20]
Whatman 903™ protein saver cards; 10 µL Mitra® Microsampling Device	6.0 mm	-	LC–MS	Gunash et al. (2019) [29]
Whatman 903™ filter paper cards	3.2 mm	-	DI-MS	Snowden et al. (2020) [19]
Whatman 903™ paper (#10,531,018, GE Healthcare)	Corresponding to 2.5 µL of blood	-	SFC-MS	Le Faouder et al. (2021) [37]
Others				
Filter papers (PKU card, Tokyo, Japan)	3.0 mm	-	GC–MS	Kimura et al. (2002) [38]
Whatman FTA DMPK-A cards	3.0 mm	-	LC–MS	Ismaiel et al. (2012) [15]
Filter paper cards Ahlstram 226, ID Biological Systems	3.2 mm	-	LC–MS	Koulman et al. (2014) [24]
✓ Fluka blood collection paper ✓ Whatman 903™ specimen collection paper ✓ Whatman 3MM chromatography paper ✓ Whatman ion exchange paper (46 × 57 cm^2)	~225 mm^2	BHT	GC–MS	Liu et al. (2014) [23]
INF	3.0 mm	-	DI-MS	Al-Thihli et al. (2014) [18]
Whatman cellulose chromatography paper strips	100 mm^2	-	LC–MS	Henao et al. (2016) [28]
Whatman FTA Classic Card	¼" diameter punch	-	LC–MS	Kyle et al. (2017) [39]
PUFAcoat™ paper	6.0 mm	-	LC–MS	Hewawasam et al. (2017) [26]

BHT, butylated hydroxytoluene; *PKU*, phenylketonuria; *DMPK*, drug metabolism and pharmacokinetics; *INF*, information not found

compared four different DBS card types: Fluka blood collection paper (Sigma-Aldrich, Switzerland); Whatman 903™ specimen collection paper; Whatman 3MM chromatography paper; and Whatman ion exchange paper (46 × 57 cm^2, Whatman, UK). Changes on the concentration of saturated fatty acids (FA), 16:0, 18:0, 22:0, and 24:0; monounsaturated FA, 16:1*n*-7, 18:1*n*-7, 18:1*n*-9, and 24:1*n*-9; and PUFA, 18:2 *n*-6, 20:3 *n*-6, 20:4 *n*-6 (arachidonic acid, AA), 22:4 *n*-6, 18:3 *n*-3, 20:5 *n*-3 (eicosapentaenoic acid, EPA), 22:5 *n*-3, and 22:6 *n*-3 (docosahexaenoic acid, DHA), were observed according with the type of paper card used and the presence/absence of antioxidants [23]. It was observed a significant decrease of PUFA in DBS samples over 4 weeks of storage at room temperature, even though the papers were impregnated with butylated hydroxytoluene (BHT, an antioxidant agent) and regardless of the DBS card type. FA composition of DBS between samples collected on Fluka blood collection paper, Whatman 3MM paper, and Whatman 903 paper did not suffer significant alterations at any time point over the storage period. However, it was noticed a lower decline in the levels of all long-chain PUFA in the DBS samples collected on the paper card Whatman ion exchange when compared to the other types of papers following 4 weeks of storage at room temperature [23]. As far as our knowledge goes, this is the only lipidomic study comparing lipid stability on different card types.

DBS cards using stabilizers

DBS paper cards are often impregnated with stabilizers or modifiers to increase analyte stability and recovery efficiency (Table 1) [1]. The addition of chemicals to DBS paper may directly influence the results due to matrix effects. Two studies performed stability analysis focusing on the efficiency of adding antioxidants to prevent lipid degradation [20, 21]. Metherel and co-workers assessed the efficiency of adding different concentrations of BHT [0 mg/mL (control), 2.5 mg/mL, and 5 mg/mL] to the DBS paper cards to prevent PUFA degradation and test its stability [21]. The study showed that BHT provides significant protection against PUFA degradation in a DBS sample, in open air and room temperature storage, and that PUFA losses are dependent on BHT concentrations. The analysis by GC–MS showed that the degradation of highly unsaturated FA (AA; EPA; DHA); *n*-3 FA; *n*-6 FA; and total PUFA differs with BHT concentrations (the ability to prevent PUFA degradation increases with higher BHT concentrations) [21]. The same PUFA are detected in DBS in the presence of BHT for up to 21 days in open air. However, total PUFA levels significantly decrease after 3 and 14 days of storage. Also, storing DBS adsorbed in BHT in sealable containers further prevents PUFA loss up to 8 weeks [21].

On the other side, Marino and colleagues validated the use of different antioxidants to prevent FA degradation after 15 days of blood collection [20]. The study revealed that, in general, antioxidants (pure or in mixtures) are not useful to accurately measure individual FA. However, the use of sodium sulphite or ascorbic acid or their mixture with BHT, ascorbic acid or gallic acid, in some cases enabled the assessment of FA. The results obtained by GC-FID showed that the levels of saturated FA (14:0, 16:0, 18:0, and 24:0) and unsaturated FA (*trans*-16:1, 16:1, *trans*-18:1, 18:1, *trans*-18:2, 18:2, α-18:3, γ-18:3, 20:1, 20:2, 20:3, AA, EPA, 22:4, 24:1, 22:5, and DHA) were different according with the antioxidant applied on the DBS paper card [20]. Although storage conditions and BHT concentration were not the same, contrarily to what was reported by Metherel et al. [21], the use of BHT in this study did not prevent FA degradation.

Matrix effects in DBS

In what concerns DBS matrix effects, these can be qualitatively assessed by analysing a blank DBS card [40], but also quantitatively determined by comparing the signals obtained from solvent extraction of a blank DBS. However, matrix effects from DBS cards are dependent on the type of analyte and can cause interference in the extraction efficiency, stability, and contribute to ionization suppression or enhancement in the analysis of the extracts from DBS by MS lipidomic approaches [41]. Phosphatidylcholines are well known as the primary cause of matrix effects in LC–MS/MS and cholesterol, cholesterol esters, and triacylglycerols, when present at relatively high concentrations can also result in significant ion suppression effects [15, 42]. An additional problem is the potential adsorption and non-specific binding of the lipids to the sampling card. DBS paper cards contain cellulose that have several -OH groups and these can interact with the hydrophilic head of the lipid molecules [43, 44]. This phenomenon has also been reported for proteins in DBS [45, 46]. When analysing samples from DBS cards, it is generally assumed that blood and analytes spread homogeneously. However, when the spread is heterogenous, which it is called chromatographic effect [1], we could have dissimilar extractions yield, depending on the punched circle of the blood card analysed. Chromatographic effects lead to significantly different results from punch to punch and it can be induced by many factors, for instance, the type of DBS card [47], Hct levels, portioning of cell components [48], and sample integrity and preparation [49]. The commercialization of DBS cards with the same chemical composition (to prevent matrix effects and the drawbacks associated), as well as the development of lipid extraction guidelines (to prevent chromatographic effects), would facilitate the dissemination of unbiased results.

Haematocrit interference

The Hct is a parameter that can affect the amount of lipids and their recovery from DBS. Hct may be considered the major factor of variability, when using DBS for metabolite analysis. Higher Hct levels are associated with increased viscosity of the blood drop, thus having a lower spread rate of the blood onto the paper card [50]. More viscous blood has been shown to have an heterogenous spread across the paper than samples with lower Hct levels, which is expected to translate into analytical biases [50–52]. The variation of the Hct value directly affects the total amount of analytes and their extraction recovery rate from DBS (lower extraction recoveries at higher Hct levels) [53]. When Hct values are high, sonication of the DBS sample has proven to be valuable to improve extraction recovery [54]. The lipidomic approaches were used in a study aiming to find lipid species' markers for quantification of Hct using DBS samples [27]. Through LC–ESI–MS, Liao et al. identified 189 lipid species belonging to PC and SM classes. The study showed that three SM, specifically SM 44:1, SM 44:2, and SM 44:3, had potential to be Hct estimation markers. The results revealed that the estimation errors for the Hct values were less than 20%, demonstrating the viability of using the identified SM markers to estimate the Hct values in DBS samples [27]. From the eligible studies in this review, the common diameter of the punch is around 3 mm. Increasing the punch diameter would be expected to increase the amount of lipid extract recovered.

Overall, DBS are a promising method for a less invasive blood collection and are being considered as a favourable method for clinical lipidomic studies. Nonetheless, there are specific factors like the size of DBS punch, effect of matrix, stability, and methods of extraction, among others, that can influence the identification and quantification of the lipids. It has been demonstrated that Hct, blood viscosity, type of DBS card, chromatographic/matrix effects, and storage conditions (temperature, humidity) can induce changes in the size of the blood spots and an irregular distribution of the sample [55]. At the present time, Hct is recognized as the most relevant factor that affects the characteristics of the blood spot, but other factor can have influence as well such as drying time, blood spreading, homogeneity, and also affects the reproducibility of the extraction procedures and further analysis [8]. To overcome these challenges, it must be considered an accurate blood spotting, possible extraction of the whole DBS (not a punch with a specific diameter), and other pre-analytical parameters that need to be optimized and harmonized like the quality of the paper card and the lipid extraction protocol (detailed in the next chapter).

Extraction of lipids from DBS

The extraction of lipids from DBS is most-often performed with organic solvents, such as methanol (MeOH), dichloromethane (CH_2Cl_2), or chloroform ($CHCl_3$), under adjusted proportions. It is important to emphasize the fact that, when being analysed by MS, the lipid signal directly reflects the amount of the initial sample, the efficiency of the extraction recovery, and matrix effects [56]. There is not a standardized protocol to extract lipids from DBS; however, it is known that, depending on the polarity and composition of the lipid specie to analyse, there is a preferential extraction with different solvents [57].

Several studies compared the extraction efficiency of different solvents (isolated and in systems) and gathered significant differences (general dry time was 3 h after blood collection and storage at room temperature) [15, 26]. The ability of different absolute organic solvents [MeOH, acetonitrile (ACN), isopropyl alcohol (IPA), CH_2Cl_2, methyl tertiary-butyl ether (MTBE), ether or *n*-hexane] to extract phosphatidylcholine (PC), cholesterol (Chol), cholesteryl esters (CE), and triacylglycerols (TG) was investigated by Ismaiel and co-workers [15]. The study determined that MeOH extracted the highest amount of PC comparing with the other organic solvents. However, when lipids were extracted from DBS with IPA and ACN, contained approximately 16.0% and 2.5% of PC levels that were found in MeOH extracts, respectively. Ether, *n*-hexane, and CH_2Cl_2 showed less than 1.0% of PC levels in the MeOH extract. Among other species, PC were also quantified to evaluate the lipid recovery rate, since it is the major phospholipid class present in blood [15]. Lyso-PC presented the same behaviour as well. Regarding Chol and CE, ether extracts contained the highest concentrations when compared to the other extraction solvents — MeOH, MTBE, *n*-hexane, IPA, ACN, and CH_2Cl_2 extracts — which contained approximately 88, 80, 70, 38, 26, and 13% of Chol and CE levels in ether extracts, respectively. Extracted TG showed the same tendency, since ether extracts presented the highest levels of the TG whereas MeOH extracts demonstrated reduced levels. The remaining organic solvents — MTBE, *n*-hexane, IPA, ACN, and CH_2Cl_2 extracts — contained approximately 89, 77, 43, 21, and 18% of TG levels that were found in ether extracts, respectively. These results showed that more apolar solvents are more efficient to extract apolar lipids (Chol, CE, and TG). Therefore, the use of MeOH extracts better PL when compared with other solvents.

In the same line, Hewawasam et al. analysed the extraction efficiency of five different extraction solvent systems (80% aqueous MeOH; 70% aqueous ACN + 12 mM ammonium formate + 0.02% acetic acid; 80% aqueous ACN; 80% aqueous ACN + 0.02% formic acid; 80%

aqueous ACN + 0.05% formic acid), but in this case, the efficiency of free PUFA recovery was evaluated by LC–MS [26]. The use of 80% aqueous MeOH resulted in a significantly higher extraction of the FA 18:2, EPA, DHA, and AA from DBS. Also, free PUFA in 80% aqueous MeOH remained stable up to 1 week of storage inside the autosampler, showing no significant changes [26].

Additionally, some studies used other strategies to increase the lipid extraction efficiency, as sonication or addition of buffers. Henao et al. determined that adding sonication/homogenization and acidification of pH steps can improve the extraction efficiency of certain lipid classes, such as phosphatidylserines (PS) [28]. Additionally, Furse et al. discovered that treating DBS paper cards with a chaeotropic buffer [guanidinium chloride (6 M) and thiourea (1.5 M)] for decoagulating blood, for 24 h prior lipid extraction, considerably improved the extraction efficiency of DBS. This study determined over 200 lipid species belonging to the classes of TG and PL [16]. Both the number and total signal intensity of lipid species were higher than DBS samples without the pre-treatment [16]. Gao and partners established a high-throughput DI-MS/MS lipidomics platform to analyse the blood lipidome from DBS samples [13]. To the extraction protocol, it was added a lithium chloride (LiCl) solution to improve the extraction efficiency of acidic lipids, prevent the degradation of plasmalogen molecular species, and decrease spectral complexity. This method was able to identify and quantify, in a single DBS sample, more than 1200 lipid molecular species belonging to PL, glycerides, glycolipids, SM, acylcarnitines, and ceramides (CER) lipid class. The lipid species identified and the class distribution using DBS were analogous to whole blood samples, but it was reported that DBS extracts contained more PL and less TG than those recovered using plasma and serum samples [13].

To sum up, different extraction solvent systems have different polarities; therefore, the extracted lipids will be in accordance with the polarity of the solvent. The studies agree that MeOH (absolute or aqueous) is the best solvent to extract PL and FA from DBS. Moreover, these studies do not compare the extraction efficiency of the traditional methods such as Bligh and Dyer [58] or Folch [59], thus, with the published data, it is not possible to conclude in fact which is the best extraction method. More studies are needed to determine the best solvent/solvent system so that the extraction efficiency/recovery is maximized. Also, a standardization and harmonization of extraction protocols (including type of DBS cards and solvent quantity) is required to allow the comparison of data between different investigations.

Lipidomics analytical methodology to screen lipids from DBS

Initially in the sixties, the analysis of DBS was made using bacterial inhibition tests for the detection of phenylketonuria in newborns. Another established technique was immunoassays, where the production of monoclonal antibodies led to development of several diagnostic kits [1]. However, immunoassays have some limitations, like a possible lack of selectivity, the rather elevated cost of reagents per sample, and the long time required for development of a new assay. Later in the nineties, MS-based approaches have emerged as a significant advance in clinical diagnosis and investigation, allowing the monitoring of several biomarkers at the same time, and were established as the detection technique in DBS analysis [60]. Lipid extracts are nowadays efficiently analysed using lipidomic approaches. Lipidomics is a field of research that is growing in a fast speed. It aims to understand lipids biological functions by describing and quantifying all lipid molecular species [61]. The sensitivity of MS instruments has significantly improved and nowadays it is common to analyse the lipid extract directly through LC–MS after its extraction or in some specific cases by DI-MS.

MS lipidomic approach

The combination of lipidomics and DBS follows the normal MS workflow (lipid extraction, data acquisition by MS approaches, and data analysis). The lipidomic analysis can be untargeted, aiming to identify and quantify as much lipid species as possible, and is often used for screening and usually for comparing different groups or biological conditions. On the other side, lipidomics can be used as a targeted approach, for the identification and quantification of specific lipids, aiming to unravel specific pathways, or profiling specific markers. The lipid identification can be done with the data-dependent MS/MS files that are matched against spectral databases and libraries search (e.g., LipidMaps and MSDial) to match known fragmentations from reference compounds. Quantification of each lipid species can be done using bioinformatics tools (e.g., MZmine and MSDial), as recently reviewed [62, 63]. Despite having some studies that consider lipidomics using GC–MS for FA profiling, the true lipidomics is based on LC–MS or DI-MS analysis. Other than identify lipid species from DBS samples, lipidomics also aims to quantify the molecular species present in each sample and disease conditions. The GC–MS or GC-FID approaches have been used combined with DBS to analyse the FA profiles [11, 12, 17, 18, 23, 26, 34]. GC–MS and GC-FID

typically require sample derivatization prior extract injection, to enable the detection of hydrophilic, non-volatile, and/or thermolabile compounds [64]. These studies mainly analysed either free or esterified FA and used different chromatographic columns as well as lipid extraction protocols, as reported in Table 2, which made the standardization and comparison for this review a rather difficult task. Thus, this reinforces the fact that there should be a normalization of the protocols regarding lipidomic analysis of DBS samples.

From the 21 studies gathered in this review, it is seen a preference for LC–MS and DI-MS approaches (10 and 5 studies, respectively), mostly due to its high sensitivity and precision (Fig. 3) [1]. Reverse phase (RP) high-performance liquid chromatography (RP-HPLC) and hydrophilic interaction liquid chromatography (HILIC-LC) were used coupled to MS in DBS lipidomic studies. HILIC-LC–MS approaches are widely used in lipidomics to analyse polar lipids like PL [65]. However, from all the eligible studies, RP columns were the most used in LC–MS (RP-LC–MS) for DBS studies.

In DI-MS, the sample of the total lipid extract is directly injected in the MS without prior chromatographic separation and can be used as a real-time monitoring method. The published works on DBS and LC/DI-MS were mainly used to identify the composition of different classes such as PL, sphingolipids, TG, and CE and, in a few cases, carnitines, acylcarnitines, or FA [13, 15–19, 22, 24, 26–29, 35, 39].

The SFC-MS approach is slowly reappearing after a period shadowed by the commercial success of LC–MS. This technique is suitable for lipid analysis and allows for a shorter separation than in LC [66]. It uses CO_2 under pressure as an eluent because CO_2 exhibits favourable properties, such as being non-flammable, chemically inert, relatively nontoxic, easy to handle, and inexpensive. The separation of oxidized lipids as well as the study of positional isomers can benefit from SFC-MS approaches due to the properties of this lipidomic approach [66]. From the eligible studies, only one study using SFC-MS matched the selection criteria and focused on method development and detection of complex lipids [37]. It allowed the identification from DBS of 500 lipid species from several classes of lipids, such as phospholipids, sphingolipids, free fatty acids, sterols, and fatty acyl-carnitines.

Lipid profile identified from DBS samples

Considering the identification of DBS lipid species, different lipid classes were analysed and identified, such as FA, PUFA [20–23, 26, 29, 36, 38], or acylcarnitines [17, 18], mainly analysed by GC–MS with a few studies by LC–MS. Other classes of complex lipids like sphingolipids [27, 35], PL, Chol, TG, prenols, CER, and sterols [13, 15, 19, 24, 25, 28, 37, 39] were analysed either by LC–MS, DI-MS, or SFC-MS. However, only one study solely reported the identification of several variables of the lipid fraction, not specifying lipid classes or species (Table 2) [16].

The lipids identified in these studies also depend a lot on the objective of the work. Some studies identify FA or PC, as major lipids, or other specific classes, for pre-analytical process optimization, as described in the previous chapter. Others identify the lipidome, as for example in studies that compare whole blood with DBS, or even some when the goal is to evaluate the applicability of DBS and lipidomics in clinical settings, as we will describe.

Lipidomics comparing DBS with whole blood or plasma/serum

DBS was compared in several studies with whole blood/plasma/serum using FA profiling or complex lipids identification, by GC–MS/GC-FID, LC–MS, DI-MS, and SFC-MS (Table 2). Most of the studies identified complex lipids from specific classes but with different levels of coverage and goals. Both neutral lipids, like TG, Chol, and CE, and PL were identified in DBS lipid extracts. Only few studies identified specific classes like PC, SM, and TG, either when testing stability or other goals like sensitivity/specificity or method optimization. Even when looking for specific lipid classes, the number of species identified per class was different between studies, mainly because in most cases, the goal of the study was not a full lipid profiling. In other studies, it was used the quantification of lipid species to evaluate the extraction recovery, or other pre-analytical parameters, as buffers, addition of antioxidants, and type of paper cards, as reported in chapter 3.

Comparison of free FA profiling in DBS and blood was done by Gunash and co-workers [29]. They have compared the free FA quantification ability of DBS with a novel wicking device designed to collect a known volume of blood (10 µL Mitra® Microsampling Device) [29]. Free FA 16:0 and 18:0 were detected on the blank DBS and after using wicking device. The concentrations of the FA 16:0 were considerably higher on the wicking device tip when compared with the 6 mm DBS punch. The presence of free FA 16:0 and 18:0 in both materials was confirmed by ultra-high-performance liquid chromatography-tandem mass spectrometer (UHPLC-MS/MS). This study proved that DBS has the FA 16:0 and FA 18:0 as contaminants; however, they are present in such low abundances (lower than their presence in blood) that do not influence the results of DBS blood analysis [29].

Acylcarnitines profiling in DBS and plasma, associated with different metabolic disorders, were assessed by Primassin and partners. They showed that free carnitine concentrations in plasma were generally 3.12 (±0.13) times higher than free carnitine concentrations in DBS samples, in

Table 2 Analysis of the selected studies according with the lipidomic parameters as extraction method, MS approach, identified lipids, and considering main goals of each study

Lipids identified	Goals	Extraction method	MS lipidomic approach	Reference
GC–MS/GC-FID				
Free FA profile (molecular species identified)	✓ Comparison between filter paper with whole blood and filter paper with serum	Acetyl chloride + 6% potassium carbonate + hexane	GC–MS with a fused silica DB-5 one	Kimura et al. (2002) [38]
FA profile (molecular species identified)	✓ Stability	Direct transesterification in 14% boron trifluoride in methanol with hexane using a convectional block heater set at 95 °C for 60 min	GC–MS with a DB-FFAP capillary column	Metherel et al. (2013) [21]
FA profile (molecular species identified)	✓ Stability ✓ Comparison between DBS, plasma, whole blood, and erythrocytes	1% (v/v) H_2SO_4 in anhydrous MeOH, heated at 70 °C for 3 h	GC-FID with a BPX70 capillary column	Liu et al. (2014) [23]
n-3 and *n*-6 long-chain PUFA profile (molecular species identified)	✓ Establishment of reference intervals ✓ Comparison between groups by gender and by age	Fingertip Bell method [68]	GLC-FID with a ZB Wax capillary column	Mashavave et al. (2016) [36]
FA profile (molecular species identified)	✓ Lipid identification ✓ Stability	MeOH + chloroform/MeOH (1:1, v/v); modified Folch	GC-FID with a BPX 70 column	Drzymała-Czyz et al. (2017) [25]
FA profile (molecular species identified)	✓ Stability	Hexane + BF_3/MeOH (14%, wt/v)	GC-FID with a SP52-60 capillary column	Marino et al. (2018) [20]
LC–MS				
Free carnitine and γ-butyrobetaine	✓ Comparison between plasma, DBS, and healthy controls	MeOH	LC–ESI–MS/MS (quattro II triple-quadrupole) with a Gilson 231XL autosampler and a Hewlett-Packard HP-1100 HPLC pump	Primassin et al. (2010) [17]
PL (PC + LPC), Chol, CE, TG	✓ Extraction efficiency ✓ Ion suppression ✓ Matrix ionization effects	MeOH; ACN; IPA; CH_3Cl_2; MTBE; ether; *n*-hexane	HPLC–MS/MS with a Luna Silica analytical column along with a Gemini C18 guard column	Ismaiel et al. (2012) [15]
TG, PL (PC + PE), SM, CE	✓ Comparison between DBS, plasma, and whole blood ✓ Lipid identification ✓ Stability	H_2O + MeOH + MTBE	DI-MS: Orbitrap using a Triversa Nanomate; LC–MS (LTQ-Orbitrap) with an Hypersil Gold C18 column	Koulman et al. (2014) [24]
Lyso-SM	✓ Lipid identification	MeOH/ACN/H_2O (80:15:5, v/v/v)	LC–MS/MS (API Qtrap 4000 mass spectrometer system) with a normal-phase silica column	Chuang et al. (2014) [35]
PL (PC + PE + PS), TG, CE (molecular species identified)	✓ Optimization of the extraction protocol ✓ Extraction efficiency ✓ Comparison between whole blood and DBS	Modified Folch [chloroform/methanol (2:1, v/v) + 0.2 M $NaHPO_4$ in ddH_2O]	UHPLC-MS Q-Orbitrap with a C18 reverse phase column	Henao et al. (2016) [28]

Table 2 (continued)

Lipids identified	Goals	Extraction method	MS lipidomic approach	Reference
FA ethyl esters (molecular species identified)	✓ Matrix effects ✓ Extraction and recovery efficiency ✓ Lipid identification	Dried dimethyl sulfoxide + *n*-heptane	LC–MS/MS (Q-Trap) with a coreshell Kinetex C8 column from Phenomenex	Luginbühl et al. (2016) [22]
PL (PC + LPC, PS), SM, TG, monoacylglycerol	✓ Stability ✓ Comparison between serum and DBS	Modified Folch [water + chloroform/methanol (2:1, v/v) at − 20 °C]	LC–MS/MS (Orbitrap-QTOF) with a Waters HSS T3 column; LC-IMS-MS	Kyle et al. (2017) [39]
Free PUFA (molecular species identified)	✓ Extraction and recovery efficiency ✓ Stability ✓ Linearity and precision of the method	80% aqueous MeOH; 70% aqueous ACN + 12 mM ammonium formate + 0.02% acetic acid; 80% aqueous ACN; 80% aqueous ACN + 0.02% formic acid; 80% aqueous ACN + 0.05% formic acid	LC–MS/MS (triple quadrupole) with an Eclipse plus C8 column	Hewawasam et al. (2017) [26]
PC, SM (molecular species identified)	✓ Lipid identification ✓ Stability ✓ Matrix effect ✓ Extraction and recovery efficiency ✓ Repeatability	MeOH	UHPLC-ESI–MS (triple quadrupole) with an Agilent ZORBAX Eclipse Plus C18 column; high resolution maXis UHR-TOF	Liao et al. (2018) [27]
FA, LPC (molecular species identified)	✓ Matrix effect ✓ Lipid identification ✓ Comparison between DBS, the novel wicking device, and whole blood	Chloroform:MeOH (2:1, v/v) + BHT + sodium phosphate buffer	GC-FID with a nitroterephathalic acid modified polyethylene glycol capillary column; UHPLC-MS (Quadrupole-Orbitrap) with a C18 Ascentis Express column	Gunash et al. (2019) [29]
DI-MS				
Acylcarnitine profile	✓ Comparison of the sensitivities and specificities of DBS and serum	MeOH	DI-MS/MS utilizing a Waters Quattro Micro tandem mass spectrometer for MRM data acquisition	Al-Thihli et al. (2014) [18]
PL, glycerides, glycolipids, SM, acylcarnitines, and Cer (molecular species identified)	✓ Stability ✓ Extraction and recovery efficiency ✓ Repeatability ✓ Comparison between DBS, plasma, whole blood, and serum	Modified Bligh & Dyer [chloroform/methanol (1:1, v/v) + LiCl solution]	QTOF-MS/MS with atmospheric-pressure chemical ionization	Gao et al. (2017) [13]
PL (PC + LPC), SM, CE, TG, diacylglycerol (molecular species identified)	✓ Precision of the method ✓ Comparison between DBS	MTBE + H_2O	Orbitrap using a Triversa Nanomate and plasma	Snowden et al. (2020) [19]
PL, TG	✓ Extraction efficiency	INF	Orbitrap using a Triversa Nanomate	Furse et al. (2020) [16]
SFC-MS				
CE, Chol, TG, free FA, Cer, SM, PC, PE, PI, PG, LPC, LPE, acylcarnitines (molecular species identified)	✓ Method development and optimization ✓ Comparison between DBS and whole blood samples	Modified Folch [water + $CHCl_3$:MeOH (50:50; v/v) + internal standard mixture]	UPC^2-QTOF with an ACQUITY UPC^2 Torus diethylamine column	Le Faouder et al. (2021) [37]

INF, information not found

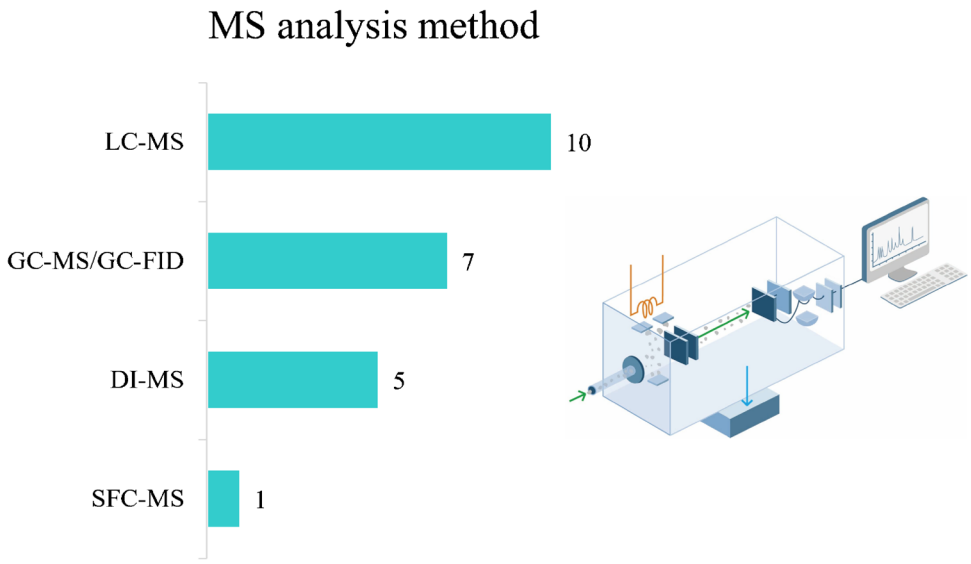

Fig. 3 Number of eligible studies of the mass spectrometry (MS) analysis method used in studies combining DBS, lipid analysis, and lipidomics. Liquid chromatography coupled with mass spectrometry (LC–MS) is the approach of choice, followed by gas chromatography coupled with mass spectrometry (GC–MS)/gas chromatography with flame ionization detection (GC-FID), direct injection-mass spectrometry (DI-MS), and lastly supercritical fluid chromatography coupled with mass spectrometry (SFC-MS)

patients with fatty acid β-oxidation disorders with and without an L-carnitine supplement [17]. In the case of patients with carnitine palmitoyltransferase I–deficiency, free carnitine concentrations were higher in DBS samples compared to plasma samples. Indeed, plasma acylcarnitine analyses have a higher sensitivity for the diagnosis of CPT2/CACT deficiencies while DBS acylcarnitine analyses have a higher sensitivity for CPT1 deficiencies [67]. Also, regarding fatty acid oxidation β-disorders, Al-Thihli et al. found by using DI-MS analysis that the sensitivity of DBS acylcarnitine profile of patients with history of rhabdomyolysis was lower than serum acylcarnitine profile [18]. DBS presented a sensitivity of 71.4%, compared to serum that presented a sensitivity of 100%. Regarding specificity, the results showed the opposite trend. DBS acylcarnitine profile had a specificity of 100% while the specificity of the serum was 94.7%. The study concluded that serum acylcarnitine profile can be more sensitive than DBS in detecting milder forms of fatty acid oxidation β-disorders, however less specific [18].

The lipid profile of plasma, whole blood, and DBS samples of healthy breast-fed infants at 3 and 12 months of age were compared using DI-MS and by LC–MS to perform lipid profile identification [24]. The lipid species identified were from the classes of TG, SM, and PC. Oxidized derivatives of CE, TG, PC, SM, and phosphatidylethanolamines (PE) were also identified. Notably, the lipid species identified in DBS samples were not different to that of whole blood samples, but as expected, both were different from plasma due to the presence of different lipid classes in whole blood. When analysing the pattern of inter-subject variability, the lipid profiles of plasma, whole blood, and DBS revealed the same trend and the results from DBS were comparable or even with better precision [24].

The composition of DBS lipidome was also compared with whole blood using untargeted lipidomic profiling, allowing the identification and quantification of lipid species of the main classes found in blood, such as PC, PE, lysophosphatidylcholine (LPC), TG, and CE [25]. The study identified, by UHPLC-MS/MS, the following acyl species: PC (16:0/18:2; 16:0/20:4; 16:0/20:5; 16:0/22:6; 18:0/20:5; 18:0/22:6; 18:1/22:6), LPC (16:0), PE (16:0/18:2; 16:0/22:6); plasmenyl PE ((P)-16:0/20:5; P-16:0/22:6; P-18:0/20:4), PS (18:0/18:1; 18:0/20:3; 18:0/20:4; 18:0/22:6), TG (16:0/18:1/18:1; 16:0/18:2/20:5; 16:0/18:2/22:6; 18:1/18:2/20:5; 16:0/18:1/22:6), and CE (18:2; 20:5; 22:6). The same lipid species were qualitatively and quantitatively identified in both samples showing that DBS can be used for comprehensive, untargeted lipidomics of the most abundant lipid species in whole blood. However, the number of identified species in this study is far from the average number of a lipidomic analysis by LC–MS, which suggests that the analysis and interpretation of the LC–MS results was limited. On the same line of work, the DBS blood lipidome was also studied by high-throughput DI-MS [26], which allowed the identification of more than 1200 lipid molecules. The lipids were extracted using a modified Bligh and Dyer extraction protocol to which was added a LiCl solution to improve the extraction efficiency of acidic lipids, prevent the degradation of plasmalogen molecular species, and decrease spectral complexity. This method was able to identify and quantify, in a single DBS sample, molecular species belonging to PL, glycerides, glycolipids, SM, acylcarnitines, and Cer lipid class. DBS results for the identified lipid species and class distribution were analogous to whole blood samples, containing more PL and less TG, as opposed to plasma and serum samples, as expected, but

similarly to blood due to the contribution of blood cells for the higher content in PL [13].

Lipidomic profiling from DBS and serum was compared, after analysis by LC–MS of samples collected in 2000–2001, to confirm lipid composition and stability of DBS and to assess if DBS would be useful time points for future use in larger cohorts of longitudinal studies of metabolic disease progression [39]. A total of 336 lipids were identified, from those, 194 were identified in the DBS and 280 were identified in the serum with 140 in common. On one side, PL were the most commonly identified lipids in both types of samples, with the greatest number of identified species belonging to PC, SM, and LPC. On the other side, monoacylglycerol and PS classes were identified only in DBS while phosphatidic acid and vitamin E were not observed in DBS samples. The presence of PS only on DBS was understandable as PS lipids are present in the lipid membrane of erythrocytes and platelets; however, PS is known to be a minor lipid class in lipoproteins. The study concludes that, since most lipid changes were preserved, DBS samples could be used in lipidomic longitudinal studies [39].

Machine learning was also used to predict 'clinical lipid' concentration from lipid profile data when compared lipid profiling from DBS with lipid profiling from plasma [19]. In this study, 118 lipid species from 11 classes were identified in DBS samples with 71% of the lipids measured in DBS also measured in plasma samples. However, the lipid classes identified were not listed in the published work. Of the 44 lipid associations from the 4 predictive panels [TG, high density lipoproteins (HDL), low density lipoprotein (LDL), and total Chol], 82% were measured in DBS samples and successfully validated with a strong correlation ($r = 0.917$) observed between the individual lipid abundance and lipoprotein concentration in both DBS and plasma samples. When applying random forest machine learning to the lipid profile data, the authors obtained good estimates ($r > 0.7$) of the concentration of TG and HDL and modest estimates ($r > 0.4$) of LDL and total Chol. However, although the obtained accuracies are significantly high, they are not suitable to be used in clinical practice, evidencing that there is room to improvement [19].

SFC-MS was used to develop a new analytical strategy for a high-throughput and comprehensive lipidomic analysis [37]. The researchers developed and optimized a new method that allowed the separation of 17 classes of lipids by SFC-MS-Q-TOF. However, when this method was applied to the evaluation of the lipidomic profile of DBS and whole blood, they could only detect 13 lipid classes (CE, Chol, TG, free FA, Cer, SM, PC, PE, phosphatidylinositol (PI), phosphatidylglycerol (PG), LPC, LPE, acylcarnitines). In the comparison of these two types of samples, the authors noticed that the relative quantities were different between whole blood samples and DBS, especially for PC, PI, PE, LPC, TG, and CE. Additionally, FA presented an unusual high level of detection, which is not typical. The qualitative profiles within each class were checked by controlling the detection of 168 different species. The molecular species profiling was very similar for almost all the classes. The authors ultimately assessed the stability of the lipids in DBS samples after 3 weeks of storage at room temperature in dark finding that the distributions of the main lipid classes within the total were similar (between the initial time and 3 weeks later), which was confirmed regarding the relative quantification of PC, PE, PI, SM, LPE, and LPC [37].

Overall, all the studies that compared whole blood with DBS proved that DBS retains the same information in lipid profiling as the analysis of whole blood since the biological matrix is the same. However, one study showed that the lipid profiling in the plasma/serum was different when compared with DBS.

DBS and lipidomics in clinical settings

Concerning the independent use of DBS method associated with lipidomics analysis in clinical settings, few papers applied the DBS method to create reference intervals, and measure diet effects and lipid levels in different pathologies comparing the results with traditional sampling methods (plasma/serum/whole blood samples) and healthy controls (Table 2) [22, 25, 35, 36, 38]. These showed that DBS are not yet established as a conventional sampling method for clinical lipidomic analysis, however, demonstrating quite promising results for example in targeted analysis of acylcarnitines in newborn screening programs.

The levels of *n*-3 and *n*-6 long-chain PUFA of DBS from Zimbabwean healthy children aged 7–9 years old were quantified by GC-FID to determine the reference interval for these FA [36]. The *n*-3 long-chain PUFA (EPA, docosapentaenoic acid *n*-3, and DHA) levels were significantly low while saturated fats, monounsaturated, and *n*-6 long-chain PUFA (AA) were surprisingly high compared to the already established reference intervals for healthy children. The 7-year-old children had lower EPA and higher AA values [36]. The low EPA and high AA levels lead to very high AA/EPA and total *n*-6 PUFA/total *n*-3 PUFA ratios, which are pro-inflammatory, thus being a health concern matter for those children.

To evaluate the effects of diet, FA esterified to glycerolipids in DBS were profiled by GC-FID and compared with the ones from venous whole blood samples in a fat challenge test [25]. In whole blood, PL are less affected, than the total lipid content, by recent dietary intake, thus are a more stable marker. The study determined that the levels of FA 18:0 esterified to PL were significantly lower in DBS when compared with whole blood. The FA 18:1 *n*-9 esterified to PL showed the opposite behaviour, with a marked increase

in DBS samples [25]. In contrast to whole blood total lipids, whole blood glycerophospholipids are less affected by recent dietary intake and thus are a more stable marker.

A method to determine FA ethyl esters, in DBS through LC–MS/MS, was developed and validated by Luginbühl and colleagues [22]. This study shows the capability of the analysis of FA ethyl esters from DBS samples as a short-term confirmation for ethanol ingestion or in the absence of traditional samples (whole blood/plasma/serum). LC–MS/MS approach proved to be advantageous compared to preliminary tests with GC–MS and solid-phase microextraction (SPME) followed by GC–MS due to its higher sensitivity and shorter run-time [22].

In the case of DBS combined with lipidomics for screening diseases, some studies reported its application to different pathologies. DBS were studied to identify markers in disease, either FA [38] or profiling polar lipid species [35]. Using GC–MS approaches, Kimura and partners analysed and compared the free FA levels of DBS with whole blood/serum from healthy controls and children with different fatty acid β-oxidation disorders [38]. Children with very long-chain acyl-CoA dehydrogenase deficiency showed a significant increase of FA 14:1. Regarding medium chain acyl-CoA dehydrogenase deficiency, FA 8:0, 10:0, and 10:1 were significantly elevated. In multiple acyl-CoA dehydrogenase deficiency, both FA 10:1 and FA 14:1 concentrations were markedly increased. In an infant fed with medium chain triglyceride milk, both FA 8:0 and FA 10:0 were increased. Children with physiological ketosis presented a slight elevation of FA 8:0 and FA 10:0 [38].

Lipid levels were also evaluated in DBS from Niemann–Pick disease type B (NPD-B) patients and compared to normal controls using LC–MS [35]. The study determined that DBS lyso-SM levels were considerably elevated in NPD-B patients when compared to normal controls, contrarily with what happens with SM levels (patient values overlapping normal controls) [35]. In this case, lyso-SM has the potential to become a biomarker of this disease, after systematic and longitudinal study of lyso-SM in clinic, evidencing the applicability of DBS sampling method for diagnosis, disease monitoring, therapeutic efficacy, and personalized medicine.

Concluding remarks and future perspectives

In summary, high-throughput lipidomics proves to be a robust and formidable tool to be applied in the analysis of DBS samples, in particular LC–MS and DI-MS. It has been demonstrated its functionality for investigation of population health and precision medicine in diverse disease states and situations.

Clinical lipidomics is an emerging line of research that focuses on the evaluation of the variation of lipids at a molecular level in several diseases. The development and improvement of MS methods for the detection of lipid molecules has become increasingly important in research field. The molecular profiling of lipids that take part of the mechanisms associated with pathophysiological processes is not yet fully understood [8]. Therefore, the main goals are to comprehend the modulation of the lipid metabolism and its mechanisms, identify new possible therapeutic targets and diagnostic biomarkers, and evaluate disease development as well as monitor disease therapeutics [12]. This review gathered information from different types of samples which can be obtained from different extraction methods and MS techniques. Hopefully in a near future, DBS can be seen as a promising approach to disease diagnostics/follow-up and therapy monitorization. Thus, the question arises: would it be useful in clinical lipidomic analysis the implementation of the DBS sampling method as a standard procedure? From the studies gathered in this review, it may be concluded that DBS are as effective as plasma/serum/whole blood samples, achieving high levels of sensitivity and specificity during MS and MS/MS analysis. The identification of the DBS lipidome through MS/MS lipidomic platforms (GC–MS, LC–MS, DI-MS, and SFC-MS) provides useful and important perceptions that empower personalized lipidomics profiling to monitor physiological changes, which could be applied to the diagnosis and follow-up of a disease [13]. However, it is necessary to pay attention to the impact of the aforementioned factors which might influence the results. When analysing lipids extracted via DBS, the possible interaction between the lipidic molecules and DBS card components can lead to ion suppression in the MS source, as well as changes in chromatographic mobility during chromatographic separation and peak sharpness. Hence, when using DBS, it must be taken into consideration the stability of the lipids on the paper cards during drying and storage, the uniformity of the blood spot, the effect of Hct, and the elution efficiency [8]. If the extraction and analysis method is already optimized, then DBS sampling would be preferable in clinical practice instead of the samples obtained by invasive venous blood collection, since drying of blood biomaterial decreases the risk of contamination with pathological and other infectious agents; and the concentration of lipids in capillary blood may differ from that in venous blood [69]. Additionally, there is a need for more studies showing the applicability of DBS in clinical lipidomics (for instance in diagnostics/follow-up of diseases), where it is independently used the DBS method in clinical settings without comparing the results with traditional samples.

In brief, the studies gathered in this review evidence that attention should be drawn to the storage and lipid extraction methods. Those methods should be made a standard

operating procedure and reinforced through quality assessments during and after implementation so that the variability is reduced, and the lipids stability is maximized to promote a successful application of DBS technology and harmonization of clinical lipidomics. Most of the published studies refer to method development and its validation with patients' samples. DBS technology is indeed the most ethical and cost-effective method of collecting, delivering, and storing the biomaterial. The DBS sampling method is a promising candidate to substitute venous blood samples offering several advantages and its application in clinical lipidomics, although still scarce, has been applied in few studies looking for disease lipids biomarkers which seems to be quite promising.

Acknowledgements Thanks are due for the financial support to the University of Aveiro and FCT/MCT for the financial support to research units CESAM (UIDB/50017/2020, UIDP/50017/2020 & LA/P/0094/2020), LAQV-REQUIMTE (UIDB/50006/2020), and CICECO-Aveiro Institute of Materials (UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020), and to RNEM, Portuguese Mass Spectrometry Network (LISBOA-01-0145-FEDER-402-022125) through national funds and, where applicable, co-financed by the FEDER, within the PT2020. Helena Beatriz Ferreira is grateful to FCT for her PhD Grant (2020.04611.BD). Inês M. S. Guerra is grateful to FCT for her PhD Grant (2021.04754.BD). Ana S. P. Moreira thanks the contract in the scope of the project 'Coccolitho4BioMat' (POCI-01-0145-FEDER-031032). Tânia Melo thanks the Junior Researcher contract in the scope of the Individual Call to Scientific Employment Stimulus 2020 (CEECIND/01578/2020). The authors are thankful to the COST Action EpiLipidNET, CA19105-Pan-European Network in Lipidomics and EpiLipidomics.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Wagner M, Tonoli D, Varesio E, Hopfgartner G. The use of mass spectrometry to analyze dried blood spots. Mass Spectrom Rev. 2016;35:361–438. https://doi.org/10.1002/mas.21441.
2. Rottinghaus EK, Beard RS, Bile E, Modukanele M, Maruping M, Mine M, Nkengasong J, Yang C. Evaluation of dried blood spots collected on filter papers from three manufacturers stored at ambient temperature for application in HIV-1 drug resistance monitoring. PLoS One. 2014;9:e109060. https://doi.org/10.1371/journal.pone.0109060.
3. Konig S, Yildiz O, Hermann N, Steurer A, Singrasa M, Dobelin W. A novel concept for sample collection and sample preparation. Int J Pharm Sci Rev Res. 2012;15:90–4.
4. Rus C-M, Di Bucchianico S, Cozma C, Zimmermann R, Bauer P. Dried blood spot (DBS) methodology study for biomarker discovery in lysosomal storage disease (LSD). Metabolites. 2021;11:382. https://doi.org/10.3390/metabo11060382.
5. Li K, Naviaux JC, Monk JM, Wang L, Naviaux RK. Improved dried blood spot-based metabolomics: a targeted, broad-spectrum, single-injection method. Metabolites. 2020;10:82. https://doi.org/10.3390/metabo10030082.
6. Nakajima D, Ohara O, Kawashima Y. Toward proteome-wide exploration of proteins in dried blood spots using liquid chromatography-coupled mass spectrometry. Proteomics. 2021;21:2100019. https://doi.org/10.1002/pmic.202100019.
7. Dhingra N, Diepart M, Dziekan G, Khamassi S, Otaiza F, Wilburn S (2010) WHO guidelines on drawing blood: best practices in phlebotomy. World Health Organization, https://www.euro.who.int/__data/assets/pdf_file/0005/268790/WHO-guidelines-on-drawing-blood-best-practices-in-phlebotomy-Eng.pdf. Accessed 14 June 2022
8. Malsagova K, Kopylov A, Stepanov A, Butkova T, Izotov A, Kaysheva A. Dried blood spot in laboratory: directions and prospects. Diagnostics. 2020;10:248. https://doi.org/10.3390/diagnostics10040248.
9. Lima-Oliveira G, Lippi G, Salvagno GL, Picheth G, Guidi GC. Laboratory Diagnostics and Quality of Blood Collection/Laboratorijska Dijagnostika I Kvalitet Uzimanja Uzoraka Krvi. J Med Biochem. 2015;34:288–94. https://doi.org/10.2478/jomb-2014-0043.
10. Lim MD. Dried blood spots for global health diagnostics and surveillance: opportunities and challenges. Am J Trop Med Hyg. 2018;99:256–65. https://doi.org/10.4269/ajtmh.17-0889.
11. Burnett JE. Dried blood spot sampling: practical considerations and recommendation for use with preclinical studies. Bioanalysis. 2011;3:1099–107.
12. Lv J, Zhang L, Yan F, Wang X. Clinical lipidomics: a new way to diagnose human diseases. Clin Transl Med. 2018;7:10–2. https://doi.org/10.1186/s40169-018-0190-9.
13. Gao F, McDaniel J, Chen EY, Rockwell HE, Drolet J, Vishnudas VK, Tolstikov V, Sarangarajan R, Narain NR, Kiebish MA. Dynamic and temporal assessment of human dried blood spot MS/MSALL shotgun lipidomics analysis. Nutr Metab (Lond). 2017;14:28. https://doi.org/10.1186/s12986-017-0182-6.
14. Wilson I. Global metabolic profiling (metabonomics/metabolomics) using dried blood spots: advantages and pitfalls. Bioanalysis. 2011;3:2255–7. https://doi.org/10.4155/bio.11.221.
15. Ismaiel OA, Jenkins RG, Thomas Karnes H. Investigation of endogenous blood lipids components that contribute to matrix effects in dried blood spot samples by liquid chromatography-tandem mass spectrometry. Drug Test Anal. 2013;5:710–5. https://doi.org/10.1002/dta.1421.
16. Furse S, Koulman A. Lipid extraction from dried blood spots and dried milk spots for untargeted high throughput lipidomics. Mol Omi. 2020;16:563–72. https://doi.org/10.1039/d0mo00102c.
17. Primassin S, Spiekerkoetter U. ESI-MS/MS measurement of free carnitine and its precursor γ-butyrobetaine in plasma and dried blood spots from patients with organic acidurias and fatty acid oxidation disorders. Mol Genet Metab. 2010;101:141–5. https://doi.org/10.1016/j.ymgme.2010.06.012.
18. Al-Thihli K, Sinclair G, Sirrs S, Mezei M, Nelson J, Vallance H. Performance of serum and dried blood spot acylcarnitine profiles for detection of fatty acid β-oxidation disorders in adult patients with rhabdomyolysis. J Inherit Metab Dis. 2014;37:207–13. https://doi.org/10.1007/s10545-012-9578-7.
19. Snowden SG, Korosi A, de Rooij SR, Koulman A. Combining lipidomics and machine learning to measure clinical lipids in dried blood spots. Metabolomics. 2020;16:83. https://doi.org/10.1007/s11306-020-01703-0.
20. Di Marino C, De Marco A, Pisanti A, Romanucci V. Effects of dried blood spot storage on lipidomic analysis. Molecules. 2018;23:403. https://doi.org/10.3390/molecules23020403.
21. Metherel AH, Hogg RC, Buzikievich LM, Stark KD. Butylated hydroxytoluene can protect polyunsaturated fatty acids in dried blood spots from degradation for up to 8 weeks at room temperature. Lipids Health Dis. 2013;12:22. https://doi.org/10.1186/1476-511X-12-22.

22. Luginbühl M, Schröck A, König S, Schürch S, Weinmann W. Determination of fatty acid ethyl esters in dried blood spots by LC–MS/MS as markers for ethanol intake: application in a drinking study. Anal Bioanal Chem. 2016;408:3503–9. https://doi.org/10.1007/s00216-016-9426-y.
23. Liu G, Mühlhäusler BS, Gibson RA. A method for long term stabilisation of long chain polyunsaturated fatty acids in dried blood spots and its clinical application. Prostaglandins, Leukot Essent Fat Acids. 2014;91:251–60. https://doi.org/10.1016/j.plefa.2014.09.009.
24. Koulman A, Prentice P, Wong MCY, Matthews L, Bond NJ, Eiden M, Griffin JL, Dunger DB. The development and validation of a fast and robust dried blood spot based lipid profiling method to study infant metabolism. Metabolomics. 2014;10:1018–25. https://doi.org/10.1007/s11306-014-0628-z.
25. Drzymała-Czyż S, Janich S, Klingler M, Demmelmair J, Walkowiak J, Koletzko B. Whole blood glycerophospholipids in dried blood spots — a reliable marker for the fatty acid status. Chem Phys Lipids. 2017;207:1–9. https://doi.org/10.1016/j.chemphyslip.2017.06.003.
26. Hewawasam E, Liu G, Jeffery DW, Muhlhausler BS, Gibson RA. A validated method for analyzing polyunsaturated free fatty acids from dried blood spots using LC–MS/MS. Prostaglandins, Leukot Essent Fat Acids. 2017;125:1–7. https://doi.org/10.1016/j.plefa.2017.08.010.
27. Liao HW, Lin SW, Lin YT, Lee CH, Kuo CH. Identification of potential sphingomyelin markers for the estimation of hematocrit in dried blood spots via a lipidomic strategy. Anal Chim Acta. 2018;1003:34–41. https://doi.org/10.1016/j.aca.2017.11.041.
28. Aristizabal Henao JJ, Metherel AH, Smith RW, Stark KD. Tailored extraction procedure is required to ensure recovery of the main lipid classes in whole blood when profiling the lipidome of dried blood spots. Anal Chem. 2016;88:9391–6. https://doi.org/10.1021/acs.analchem.6b03030.
29. Gunash J, Aristizabal-Henao JJ, Stark KD. Quantitating fatty acids in dried blood spots on a common collection card versus a novel wicking sampling device. Prostaglandins, Leukot Essent Fat Acids. 2019;145:1–6. https://doi.org/10.1016/j.plefa.2019.05.002.
30. Li W, Zhang J, Tse FLS. Strategies in quantitative LC-MS/MS analysis of unstable small molecules in biological matrices. Biomed Chromatogr. 2011;25:258–77. https://doi.org/10.1002/bmc.1572.
31. Grüner N, Stambouli O, Ross RS. Dried blood spots—preparing and processing for use in immunoassays and in molecular techniques. J Vis Exp. 2015;13:52619. https://doi.org/10.3791/52619.
32. Ho NT, Busik JV, Resau JH, Paneth N, Khoo SK. Effect of storage time on gene expression data acquired from unfrozen archived newborn blood spots. Mol Genet Metab. 2016;119:207–13. https://doi.org/10.1016/j.ymgme.2016.08.001.
33. Pupillo D, Simonato M, Cogo PE, Lapillonne A, Carnielli VP. Short-term stability of whole blood polyunsaturated fatty acid content on filter paper during storage at −28 °C. Lipids. 2016;51:193–8. https://doi.org/10.1007/s11745-015-4111-z.
34. Han J, Higgins R, Lim MD, Lin K, Yang J, Borchers CH. Short-term stabilities of 21 amino acids in dried blood spots. Clin Chem. 2018;64:400–2. https://doi.org/10.1373/clinchem.2017.278457.
35. Chuang WL, Pacheco J, Cooper S, McGovern MM, Cox GF, Keutzer J, Zhang XK. Lyso-sphingomyelin is elevated in dried blood spots of Niemann-Pick B patients. Mol Genet Metab. 2014;111:209–11. https://doi.org/10.1016/j.ymgme.2013.11.012.
36. Mashavave G, Kuona P, Tinago W, Stray-Pedersen B, Munjoma M, Musarurwa C. Dried blood spot omega-3 and omega-6 long chain polyunsaturated fatty acid levels in 7–9 year old Zimbabwean children: a cross sectional study. BMC Clin Pathol. 2016;16:14. https://doi.org/10.1186/s12907-016-0035-7.
37. Le Faouder P, Soullier J, Tremblay-Franco M, Tournadre A, Martin J-F, Guitton Y, Carlé C, Caspar-Bauguil S, Denechaud P-D, Bertrand-Michel J. Untargeted lipidomic profiling of dry blood spots using SFC-HRMS. Metabolites. 2021;11:305. https://doi.org/10.3390/metabo11050305.
38. Kimura M, Yoon HR, Wasant P, Takahashi Y, Yamaguchi S. A sensitive and simplified method to analyze free fatty acids in children with mitochondrial beta oxidation disorders using gas chromatography/mass spectrometry and dried blood spots. Clin Chim Acta. 2002;316:117–21. https://doi.org/10.1016/S0009-8981(01)00741-0.
39. Kyle JE, Casey CP, Stratton KG, Zink EM, Kim Y, Zheng X, Monroe ME, Weitz KK, Bloodsworth KJ, Orton DJ, Ibrahim YM, Moore RJ, Lee CG, Pedersen C, Orwoll E, Smith RD, Burnum-Johnson KE, Baker ES. Comparing identified and statistically significant lipids and polar metabolites in 15-year old serum and dried blood spot samples for longitudinal studies. Rapid Commun Mass Spectrom. 2017;31:447–56. https://doi.org/10.1002/rcm.7808.
40. Liu G, Patrone L, Snapp HM, Batog A, Valentine J, Cosma G, Tymiak A, Ji QC, Arnold ME. Evaluating and defining sample preparation procedures for DBS LC–MS/MS assays. Bioanalysis. 2010;2:1405–14. https://doi.org/10.4155/bio.10.106.
41. Clark GT, Haynes JJ. Utilization of DBS within drug discovery: a simple 2D-LC–MS/MS system to minimize blood- and paper-based matrix effects from FTA elute™ DBS. Bioanalysis. 2011;3:1253–70. https://doi.org/10.4155/bio.11.81.
42. Ismaiel OA, Zhang T, Jenkins RG, Karnes HT. Investigation of endogenous blood plasma phospholipids, cholesterol and glycerides that contribute to matrix effects in bioanalysis by liquid chromatography/mass spectrometry. J Chromatogr B. 2010;878:3303–16. https://doi.org/10.1016/j.jchromb.2010.10.012.
43. Gurtovenko AA, Mukhamadiarov EI, Kostritskii AY, Karttunen M. Phospholipid–cellulose interactions: insight from atomistic computer simulations for understanding the impact of cellulose-based materials on plasma membranes. J Phys Chem B. 2018;122:9973–81. https://doi.org/10.1021/acs.jpcb.8b07765.
44. Kostritskii AY, Tolmachev DA, Lukasheva NV, Gurtovenko AA. Molecular-level insight into the interaction of phospholipid bilayers with cellulose. Langmuir. 2017;33:12793–803. https://doi.org/10.1021/acs.langmuir.7b02297.
45. Skjærvø Ø, Solbakk EJ, Halvorsen TG, Reubsaet L. Paper-based immunocapture for targeted protein analysis. Talanta. 2019;195:764–70. https://doi.org/10.1016/j.talanta.2018.12.013.
46. McCann L, Benavidez TE, Holtsclaw S, Garcia CD. Addressing the distribution of proteins spotted on μPADs. Analyst. 2017;142:3899–905. https://doi.org/10.1039/C7AN00849J.
47. Cobb Z, de Vries R, Spooner N, Williams S, Staelens L, Doig M, Broadhurst R, Barfield M, van de Merbel N, Schmid B, Siethoff C, Ortiz J, Verheij E, van Baar B, White S, Timmerman P. In-depth study of homogeneity in DBS using two different techniques: results from the EBF DBS-microsampling consortium. Bioanalysis. 2013;5:2161–9. https://doi.org/10.4155/bio.13.171.
48. Stickle DF, Rawlinson NJ, Landmark JD. Increased perimeter red cell concentration in filter paper bloodspot samples is consistent with constant-load size exclusion chromatography occurring during application. Clin Chim Acta. 2009;401:42–5. https://doi.org/10.1016/j.cca.2008.11.011.
49. Kvaskoff D, Ko P, Simila HA, Eyles DW. Distribution of 25-hydroxyvitamin D3 in dried blood spots and implications for its quantitation by tandem mass spectrometry. J Chromatogr B. 2012;901:47–52. https://doi.org/10.1016/j.jchromb.2012.05.040.
50. Hall E, Flores S, De Jesús V. Influence of hematocrit and total-spot volume on performance characteristics of dried blood spots for newborn screening. Int J Neonatal Screen. 2015;1:69–78. https://doi.org/10.3390/ijns1020069.

51. De Kesel PM, Sadones N, Capiau S, Lambert WE, Stove CP. Hemato-critical issues in quantitative analysis of dried blood spots: challenges and solutions. Bioanalysis. 2013;5:2023–41. https://doi.org/10.4155/bio.13.156.
52. Chao TC, Trybala A, Starov V, Das DB. Influence of haematocrit level on the kinetics of blood spreading on thin porous medium during dried blood spot sampling. Colloids Surf A Physicochem Eng Asp. 2014;451:38–47. https://doi.org/10.1016/j.colsurfa.2014.03.033.
53. Youhnovski N, Bergeron A, Furtado M, Garofolo F. Pre-cut dried blood spot (PCDBS): an alternative to dried blood spot (DBS) technique to overcome hematocrit impact. Rapid Commun Mass Spectrom. 2011;25:2951–8. https://doi.org/10.1002/rcm.5182.
54. Li F, Ploch S, Fast D, Michael S. Perforated dried blood spot accurate microsampling: the concept and its applications in toxicokinetic sample collection. J Mass Spectrom. 2012;47:655–67. https://doi.org/10.1002/jms.3015.
55. Ren X, Paehler T, Zimmer M, Guo Z, Zane P, Emmons GT. Impact of various factors on radioactivity distribution in different DBS papers. Bioanalysis. 2010;2:1469–75. https://doi.org/10.4155/bio.10.96.
56. Trufelli H, Palma P, Famiglini G, Cappiello A. An overview of matrix effects in liquid chromatography–mass spectrometry. Mass Spectrom Rev. 2011;30:491–509. https://doi.org/10.1002/mas.20298.
57. Marsh D. Handbook of lipids bilayers. 2nd ed. Boca Raton, FL, USA: CRC Press; 2013.
58. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 1959;37:911–7. https://doi.org/10.1139/o59-099.
59. Eggers LF, Schwudke D (2016) Liquid extraction: Folch. Encycl Lipidomics 1–6https://doi.org/10.1007/978-94-007-7864-1_89-1
60. Chace DH, Kalas TA, Naylor EW. Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns. Clin Chem. 2003;49:1797–817. https://doi.org/10.1373/clinchem.2003.022178.
61. O'Donnell VB, Ekroos K, Liebisch G, Wakelam M. Lipidomics: current state of the art in a fast moving field. WIREs Syst Biol Med. 2020;12:e1466. https://doi.org/10.1002/wsbm.1466.
62. Züllig T, Trötzmüller M, Köfeler HC. Lipidomics from sample preparation to data analysis: a primer. Anal Bioanal Chem. 2020;412:2191–209. https://doi.org/10.1007/s00216-019-02241-y.
63. Alves MA, Lamichhane S, Dickens A, McGlinchey A, Ribeiro HC, Sen P, Wei F, Hyötyläinen T, Orešič M. Systems biology approaches to study lipidomes in health and disease. Biochim Biophys Acta - Mol Cell Biol Lipids. 2021;1866:158857. https://doi.org/10.1016/j.bbalip.2020.158857.
64. Domingues P, García A, Skrzydlewska E (2018) Advanced analytical chemistry for life sciences. AACLifeSci, https://www.umb.edu.pl/photo/pliki/projekty_umb/aac/aaclifesci_-_manual.pdf. Accessed 14 June 2022
65. Li A, Hines KM, Xu L. Lipidomics by HILIC-ion mobility-mass spectrometry. Methods Mol Biol. 2020;2084:119–32. https://doi.org/10.1007/978-1-0716-0030-6_7.
66. Laboureur L, Ollero M, Touboul D. Lipidomics by supercritical fluid chromatography. Int J Mol Sci. 2015;16:13868–84. https://doi.org/10.3390/ijms160613868.
67. de Sain-van der Velden MGM, Diekman EF, Jans JJ, van der Ham M, Prinsen BHCMT, Visser G, Verhoeven-Duif NM (2013) Differences between acylcarnitine profiles in plasma and bloodspots. Mol Genet Metab 110:116–121.https://doi.org/10.1016/j.ymgme.2013.04.008.
68. Gordon Bell J, Mackinlay EE, Dick JR, Younger I, Lands B, Gilhooly T. Using a fingertip whole blood sample for rapid fatty acid measurement: method validation and correlation with erythrocyte polar lipid compositions in UK subjects. Br J Nutr. 2011;106:1408–15. https://doi.org/10.1017/S0007114511001978.
69. Mohammed BS, Cameron GA, Cameron L, Hawksworth GH, Helms PJ, McLay JS. Can finger-prick sampling replace venous sampling to determine the pharmacokinetic profile of oral paracetamol? Br J Clin Pharmacol. 2010;70:52–6. https://doi.org/10.1111/j.1365-2125.2010.03668.x.