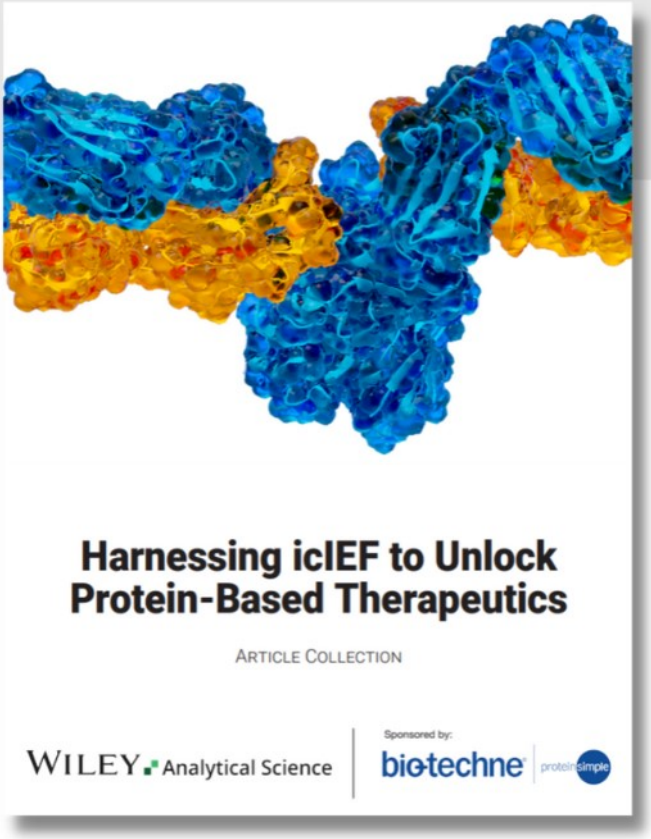
Harnessing icIEF to Unlock Protein-Based Therapeutics



Read the new Article Collection

Keep up to date with the latest developments in biotherapeutics and the range of treatments for various diseases with our latest article collection. Find out how imaged cIEF (icIEF) technique is essential for quality control and analytical development of these drugs, as it accurately determines the surface charge of lipid nanoparticles and the charge heterogeneity of proteins and antibodies.

This article collection aims to provide you with more information on these techniques and technologies, helping you further your research in this field.

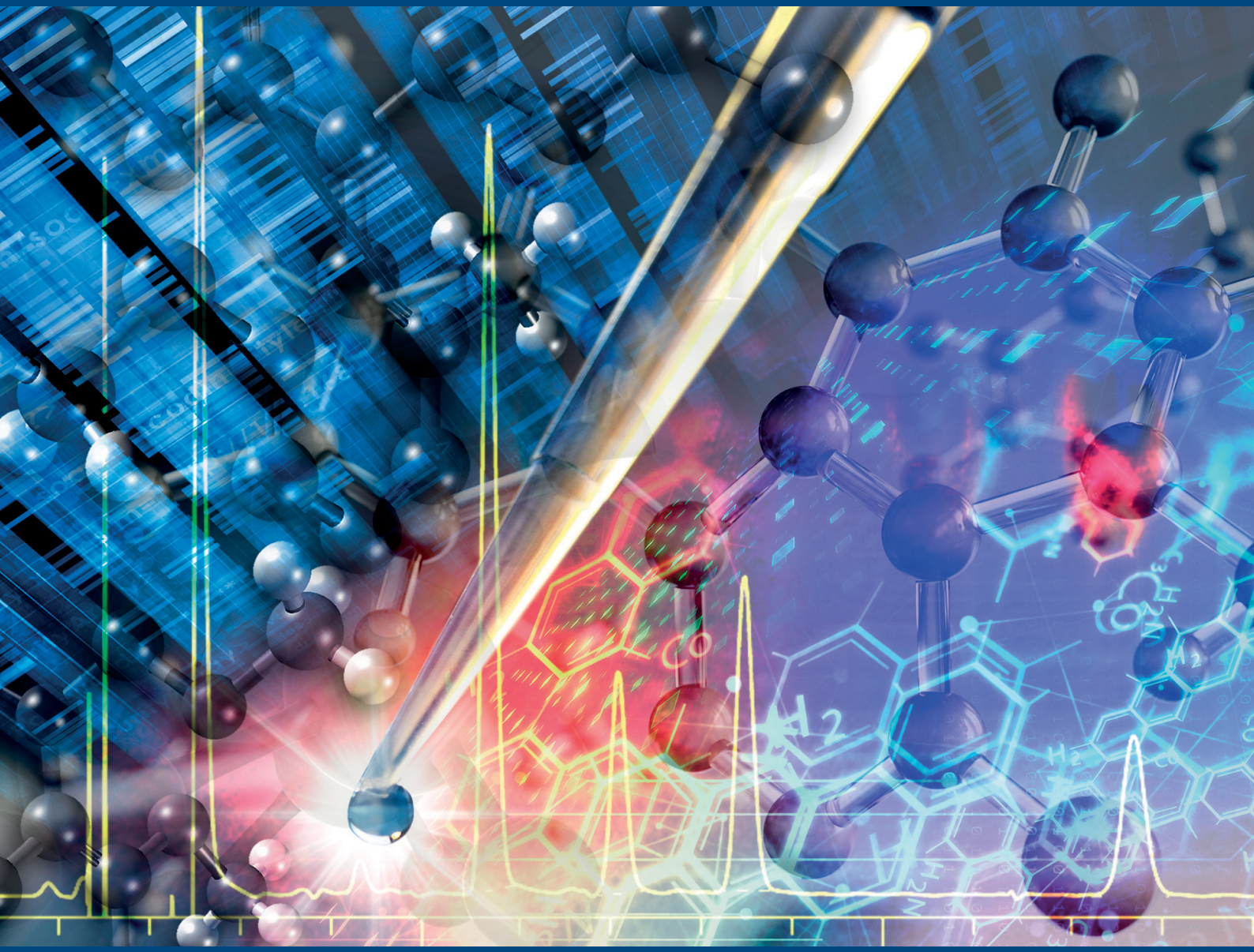
bio-techne® | protein simple

WILEY

ISSN 1615-9306 · JSSCCJ 46 (15) 2023 · Vol. 46 · No. 15 · August 2023

Journal of SEPARATION SCIENCE

15|2023



www.jss-journal.com

Methods
Chromatography · Electroseparation

Applications
Biomedicine · Foods · Environment

WILEY VCH

Received: 30 March 2023 | Revised: 11 May 2023 | Accepted: 12 May 2023

DOI: 10.1002/jssc.202300210

JOURNAL OF SEPARATION SCIENCE

REVIEW ARTICLE

Dried blood spot analysis with liquid chromatography and mass spectrometry: Trends in clinical chemistry

Hanne Bendiksen Skogvold[1,2] | **Helge Rootwelt**[1] | **Léon Reubsaet**[3] | **Katja Benedikte Prestø Elgstøen**[1] | **Steven Ray Wilson**[4,5]

[1]Department of Medical Biochemistry, Oslo University Hospital, Rikshospitalet, Oslo, Norway

[2]Department of Mechanical, Electronic and Chemical Engineering, Oslo Metropolitan University, Oslo, Norway

[3]Department of Pharmacy, Section of Pharmaceutical Chemistry, University of Oslo, Oslo, Norway

[4]Department of Chemistry, University of Oslo, Oslo, Norway

[5]Hybrid Technology Hub – Centre of Excellence, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Oslo, Norway

Correspondence
Steven Ray Wilson, Department of Chemistry, University of Oslo, Post Box 1033, Blindern, NO-0315 Oslo, Norway.
Email: stevenw@kjemi.uio.no

Dried blood spot samples are simple to prepare and transport, enabling safe and accessible diagnostics, both locally and globally. We review dried blood spot samples for clinical analysis, focusing on liquid chromatography-mass spectrometry as a versatile measurement tool for these samples. Dried blood spot samples can provide information for, for example, metabolomics, xenobiotic analysis, and proteomics. Targeted analyses of small molecules are the main application of dried blood spot samples and liquid chromatography-mass spectrometry, but emerging applications include untargeted metabolomics and proteomics. Applications are highly varied, including analyses related to newborn screening, diagnostics and monitoring of disease progression and treatment effects of virtually any disease, as well as studies into the physiology and effects of diet, exercise, xenobiotics, and doping. A range of dried blood spot products and methods are available, and applied liquid chromatography-mass spectrometry instrumentation is varied with regard to liquid chromatography column formats and selectivity. In addition, novel approaches such as on-paper sample preparation (e.g., selective trapping of analytes with paper-immobilized antibodies) are described. We focus on research papers published in the last 5 years.

KEYWORDS
dried blood spot, liquid chromatography, mass spectrometry, omics, xenobiotics

1 | INTRODUCTION

1.1 | A global need for simple sampling and sample handling in clinical diagnostics

In vast regions of the world, a large proportion of the population does not even have access to the most basic types of medical care, reflecting an unacceptable inequality in health care delivery. Correct diagnosis is a cornerstone of all evidence-based medical treatments. Diagnostics based

Article Related Abbreviations: ASD, autism spectrum disorder; DBS, dried blood spot; DIA, data independent analysis; Hct, hematocrit; HILIC, hydrophilic interaction liquid chromatography; HRMS, high-resolution mass spectrometry; mAb, monoclonal antibody; MRM, multiple reaction monitoring; NBS, newborn screening; TDM, therapeutic drug monitoring.

on clinical investigations require the presence of a physician or another educated health professional. But even the best of doctors, if available, depend on laboratory analyses for sufficient diagnostic precision and quantitative measurements of health, disease, and effect of treatment. Standard laboratory diagnostics typically require analysis using relatively advanced instrumentation in controlled environments. In order to obtain adequate diagnostics for the treatment needed, the patient must have access to a healthcare professional, adequate laboratory sampling facilities, and time-limited and temperature-controlled transportation of the sample to the laboratory. As of now, this is out of reach for many.

1.2 | Dried blood spot samples: a tool for accessible and safe clinical diagnostics

Within-reach approaches to healthcare are therefore of paramount importance. One such approach, which we here focus on, is simple at-home finger-prick-based blood sampling onto filter paper dried blood spots (DBS), see Figure 1. DBS can provide sufficient sample stability of at least a week for many relevant analytes, allowing ambient temperature transportation to a centralized specialist laboratory anywhere in the world. Add to this, the era of -omics technology enables analysis of, for example, the proteome and metabolome using these dried sample materials. This allows for cost-efficient diagnostics and monitoring of treatment from virtually any outskirt on our planet. Not only will this apply to the rural areas of the world, but DBS procedures also enable at-home sampling for anyone based on necessity or mere convenience. The list of advantages is long; microsampling procedures like DBS (and other related variants, such as volumetric absorptive microsampling) are simple, minimally invasive and preferred by patients, research trial participants, and in animal research. They are suitable for self-sampling, enabling patients, relatives or caregivers to collect the sample needed at the most relevant time points in the most convenient setting, usually at home. Furthermore, the ease of sample handling, requiring only a certain time period of air drying before sealing, and cost-efficient ambient temperature shipment and storage, can provide laboratory services to all parts of the world and to all individuals who seek this service.

The feasibility of this approach has long been established through newborn screening (NBS) services where DBS are taken and shipped from rural areas and sent to specialist laboratories in other continents for analyses. Another advantage relevant to our new pandemic-focused state of mind is the obvious benefit of contactless sampling. The patient can take the necessary biological sample

FIGURE 1 "It's an insanely inequitable world. The number of people that climate is killing right now isn't 1% compared to global health inequity" (Bill Gates, Bill & Melinda Gates Foundation [2]). Can dried blood spot sampling, where a finger-prick blood sample is dripped onto a filter paper, contribute to reducing the unacceptable global health inequality with respect to laboratory diagnostics and treatment?

at home and thus obliviate the risk of contracting or spreading infections by queuing up at the local health center [1]. Therefore, DBS has both local and global advantages.

1.3 | Review objective

In this review, we will give an overview of DBS analysis in clinical applications. We will provide details on materials and sample preparation procedures for DBS. We describe approaches for the analysis of these samples, with emphasis on LC-MS (following a justification for this focus). We provide some brief historical context, while the review will primarily focus on publications from the last 5 years. We will dedicate sections to targeted metabolomics (the most established DBS/LC-MS clinical application), global metabolomics, xenobiotics (illegal drugs, doping, etc.), targeted proteomics, and global proteomics. The focus will be on applications seeing clinical usage today, but we will also

dedicate attention to novel approaches to DBS technology, that could be routinely applied in the future. We then discuss the pros and cons of the approach and try to make some predictions regarding the future of DBS/LC-MS for clinical use.

2 | DESCRIPTION OF DBS, LC, AND MS

2.1 | DBS: materials and procedures

DBS were introduced in 1913 by Ivar Christian Bang for the monitoring of glucose in rabbits [3]. In 1963, Robert Guthrie and Ada Susi presented a DBS method for phenylalanine as a biomarker for phenylketonuria, which was the origin of NBS [4]. Guthrie emphasized the importance of DBS for the development of NBS, which is now done in many countries worldwide to detect and initiate treatment for a wide range of inborn disorders, referring to DBS as his "most important contribution" [5, 6]. Another example of the early use of DBS was the measurement of free fatty acids using MS in 1976 by Mee et al. [7]. DBS were mostly used for NBS and infection diagnostics until the middle of the 2000s when its use expanded to other areas, for example, metabolomics and therapeutic drug monitoring (TDM) [8]. As reviewed by Wagner et al., the expansion of MS based techniques, especially combined with electrospray ionization (ESI), was of particular importance for the growing use of DBS [9].

The use of DBS has been extensively reviewed [9–13]. The filter cards used can be chemically treated, for example, to preserve DNA (see Table 1 for a list of common commercially available filter cards). However, the use of untreated filter cards appears to be more common, with many users employing the Whatman 903 filter card [14–16]. In fact, the only two US Food and Drug Administration-approved filter cards are the Whatman 903 and PerkinElmer 226 variants.

DBS are typically prepared by dripping blood, either directly by finger- or heel-prick, or by pipetting from a whole blood sample, onto the filter card. The blood is left to dry, usually at ambient temperatures, for a few hours (at least four hours is recommended for complete drying) [8]. The whole DBS spot can be analyzed, or a manual or automatic puncher can be used to punch out a small part of the spot, usually with a diameter of 3.2 or 6.4 mm depending on the quantitative need. For the analysis of small molecules (e.g., drugs and metabolites), the DBS or punch is typically extracted with organic solvents such as methanol or acetonitrile during shaking at room temperature or controlled heating. The extract can be dried (using e.g., nitrogen) and reconstituted in a suitable solvent, for example, the mobile phase, or injected directly, with or without centrifugation and/or filtration steps [17–22]. In contrast to the common approach of organic solvent extraction for small molecules, Gjelstad and co-workers investigated biopolymer-based cards, made of alginate and chitosan foams. The biopolymers and blood spots are dissolved in an acidic aqueous solution [23]. However, the concept has not been commercialized. In general, for protein determinations and proteomic analyses, mainly aqueous extraction solvents are used. Conventional solvents like ammonium bicarbonate buffers can be used for tryptic digestion of most of the proteins [24, 25]. For hydrophobic proteins, sodium bicarbonate can be used for solubilization and consecutive precipitation before bottom-up treatment [26]. If the DBS sample is not subject to analysis within a week, the sample is typically stored in a sealed plastic bag containing desiccant at −20 or −70°C or colder for optimal conservation.

The hematocrit (Hct) is the volume percentage of red blood cells in whole blood. Hct affects the spreading of a DBS as a higher Hct means that the blood is more viscous, thereby spreading less than a DBS with a lower Hct. Consequently, a punch of a DBS with a higher Hct will contain more whole blood than a punch of a DBS with a smaller Hct, hence affecting the quantification of an analyte. Furthermore, the migration and relative distribution of the blood constituents within the area of the DBS is affected, resulting in slightly different contents in the central vs. the peripheral parts of the DBS. Hct has also been shown to affect the recovery of analytes as well as differences in the eluate matrix effects. (See [27] for a review of the Hct effect). A DBS typically contains 50–100 µl whole blood [28, 29]. Although standard liquid venous blood samples provide the advantages of larger blood volumes and elimination of the Hct effect, there are several advantages with DBS. As the blood is dried, most biological processes, such as enzymatic reactions, stop. Furthermore, drying inactivates most microbes and viruses. Taken together, DBS is easy to obtain, suitable for simple transport and storage, biologically stable, and less biohazardous than whole blood.

2.2 | LC-MS: a versatile and sensitive tool for DBS analysis

Mass spectrometry is highly recognized for its sensitivity and selectivity, allowing for low risks of false positives and negatives when operating within the boundaries of a validated method. With MS, the exact mass of the analyte is determined, often followed by a fragmentation of the analyte, and finally mass determination of these fragments. The monitoring of such reactions allows for highly confident identifications compared to other common detection

TABLE 1 An overview of common commercially available types of filter cards for dried blood spots.

Name	Produced by	Chemically treated?	Properties	Applications	References
Whatman 903 Proteinsaver	Cytiva	No	Pure cotton linters, no additives.	Protein and metabolite analysis, global LC-MS/MS metabolomics of samples from pregnant women living with HIV, LC-MS/MS analysis of steroids	[15, 26]
PerkinElmer 226	PerkinElmer	No	Pure cotton linters, no additives.	Newborn screening (metabolite analysis), LC-MS/MS analysis of immunosuppressants, global LC-MS metabolomics of archived newborn screening samples	[27, 28]
QIAcard FTA Classic (formerly Whatman (Cytiva))	QIAGEN	Yes	Proteins are denatured. Cells are lysed. Nucleic acids are preserved.	DNA and metabolite analysis, LC-MS/MS analysis of lipids, LC-MS/MS analysis of psychoactive substances in postmortem samples	[29, 30]
QIAcard FTA DMPK-A (formerly Whatman (Cytiva))	QIAGEN	Yes	Proteins are denatured. DNA is stabilized. Cells are lysed. 20% smaller blood spot area than DMPK-B and DMPK-C.	LS-MS/MS analysis of trimethylamine N-oxide and related compounds	[31]
QIAcard FTA DMPK-B (formerly Whatman (Cytiva))	QIAGEN	Yes	Proteins are denatured. DNA is stabilized. Cells are lysed.	LC-MS/MS analysis of meropenem	[32]
QIAcard FTA DMPK-C (formerly Whatman (Cytiva))	QIAGEN	No	Better suited for protein analysis than DMPK-A and DMPK-B as proteins are not denatured.	LC-MS/MS analysis of antiepileptic drugs, LC-MS/MS analysis of clenbuterol	[22, 33]

devices, such as those based on UV-spectroscopy. Furthermore, measurements can be performed in untargeted mode (e.g., data-dependent or data-independent acquisition [DIA]), or targeted mode, that is, choosing analytes on beforehand to maximize sensitivity (e.g., selected reaction monitoring = SRM or multiple reaction monitoring [MRM]).

See Figure 2 for examples of mass spectrometry and separation systems for DBS analysis. MS can be performed "stand-alone" for DBS analysis, via, for example, direct infusion [30, 31] or MALDI [32, 33]. It is, however, often advantageous to perform a separation step of the analytes prior to MS-based analysis. Separation allows for analytes to enter the MS instruments at differing time points (retention/migration times), providing a feature for identification (e.g., two isomers from a DBS spot may share MS/MS features but not the same retention/migration time). In addition, separation can reduce sensitivity issues that can occur when many compounds enter the MS simultaneously (e.g., suppression effects when applying a common ESI interface).

Gas chromatography is readily coupled with MS, often via electron ionization (EI) interfaces [34, 35]. Spectra obtained with GC-EI-MS are typically highly reproducible and can be analyzed using established search libraries. In addition, GC can have excellent resolving power, especially when performed two-dimensionally (GC × GC [36]). However, GC separations must occur in the gas phase at up to several hundred degrees, which is incompatible with many biomolecules.

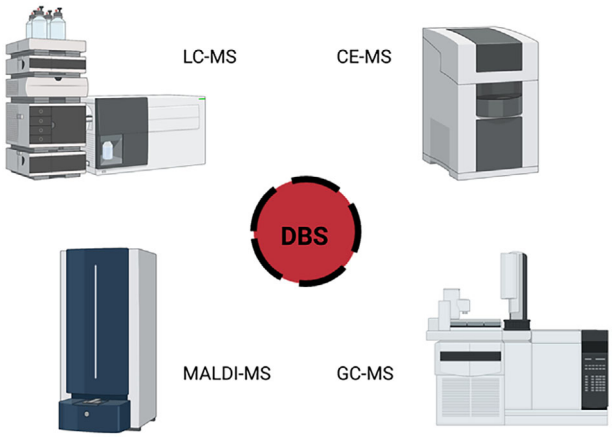


FIGURE 2 Examples of mass spectrometry and separation systems for dried blood spot (DBS) analysis. LC-MS provides versatile and robust options for analyzing most biomolecules, including polar metabolites and proteins. CE-MS provides highly efficient separations but is still considered less robust than LC-MS. MALDI-MS allows for rapid analysis but can have limitations in the comprehensive analysis as no separation step is included. GC-MS is robust and has excellent separation properties but is less suited for thermolabile biomolecules.

Several other separation variants are possible to couple with mass spectrometry-based analysis of DBS samples, including capillary electrophoresis [37, 38], and supercritical fluid chromatography [39]. These variants can provide rapid, high-resolution separations, but are less applied, perhaps related to (sometimes perceived?) limitations regarding robustness and versatility.

LC is compatible with separating drugs, polar metabolites, lipids, peptides, proteins, and other biomolecules (separations typically occur around 25–50°C). The instrumentation is robust for clinical applications and enjoys a high degree of versatility. For example, several orthogonal separation principles can be applied to DBS analysis, including RP, which is the most common, and hydrophilic interaction LC (HILIC) [40]. RPLC separates compounds according to hydrophobicity. HILIC is often associated with the separation of hydrophilic compounds but can also be used for proteomics and lipidomics. In addition, there are emerging variants for bioanalytical LC-MS, for example, ion exchange LC, which can be compatible with MS detection [41], but is virtually unused for DBS analysis so far.

LC columns are also available in various sizes and dimensions; DBS analysis of small molecules (drugs, metabolites, etc.) is typically performed using conventional bore columns (2.1–4.6 mm inner diameter), while proteomics experiments may also employ far narrower variants, for example, nano-LC (< 100 μm inner diameter).

LC-ESI-MS can also be used for a wide range of compounds, but some analytes may require derivatization to be chargeable (a requisite for ESI). Proteins can often be monitored directly (top-down proteomics), but sensitivity and identifications may be enhanced if enzymatic protein cleavage is performed and peptide products are measured (bottom-up proteomics, the most common approach in todayt's proteomics). Additional add-ons can include online SPE. Online SPE-LC can allow automated sample clean-up for more robust analysis and to reduce matrix effects, also for DBS samples [42].

Taken together, LC-MS is a versatile and robust approach for DBS analyses and has become the main approach for clinical DBS analysis. On the other hand, LC-MS instrumentation is expensive, requires specialized operator skills, and can be time-consuming compared to, for example, enzyme-linked immunosorbent assay analyses, which can be rapid, sensitive, and reasonably selective. But compared to enzyme-linked immunosorbent assay, LC-MS has a strong advantage in that it performs multianalyte measurements (up to several thousands) within the same recording of a minute sample volume. As we will see, this is particularly advantageous for global/untargeted analysis, for example, metabolomics and proteomics.

3 | CLINICAL APPLICATIONS OF DBS, LC, AND MS

3.1 | Small molecules

3.1.1 | Targeted metabolite analysis also known as "targeted metabolomics"

A challenge with NBS programs is the occurrence of false-positive results. To overcome this issue, second-tier tests are used, in which one or more separate analyses are performed to verify or dismiss the diagnostic finding in the first-tier test. Combining several markers in one second-tier test is both time- and resource-saving compared to a sequential testing protocol. It reduces delays and provides faster diagnosis or reassurance to worried parents. Using HILIC (an Imtakt Intrada amino acid column) with a Waters TQ-XS triple quadrupole MS, Kilgore et al. developed and validated a LC-MS/MS second-tier NBS method for 19 biomarkers (comprising 11 disorders), which differ widely both regarding chemical class and physiochemical properties, for example, amino acids, acylcarnitines and lipids; see reference [43] and Figure 3. Another second-tier LC-MS/MS NBS method was developed and validated by Zhan et al. for congenital adrenal hyperplasia [20]. A Waters Acquity HSS T3 column with a Waters Xevo

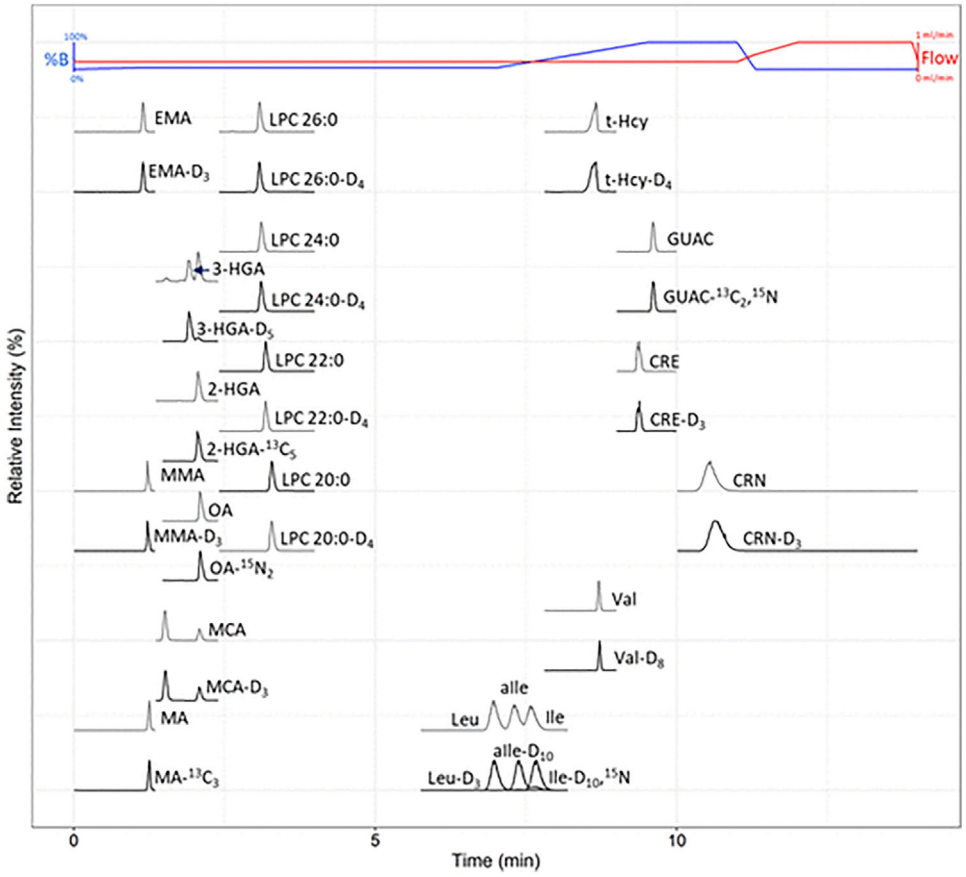


FIGURE 3 A targeted hydrophilic interaction LC (HILIC) MS/MS second-tier newborn screening method developed by Kilgore et al. was demonstrated to cover and chromatographically separate a wide range of metabolites. Reprinted [43] with permission under the Creative Commons Attribute licensing.

TQ-S triple quadrupole MS was used for the simultaneous detection of six steroids in DBS. Compared with two conventional second-tier methods for congenital adrenal hyperplasia, the positive predictive value was increased from 1.4% and 4.3% to 26.0% for the developed steroid profiling method.

Schultz et al. developed and validated an LC-MS/MS DBS method for metabolites relevant for treatment monitoring of patients with hereditary tyrosinemia type 1 (tyrosine, phenylalanine, methionine, succinylacetone and nitisinone) [44]. An Agilent Poroshell EC-C18 column with a Sciex API 3200 triple quadrupole was used. Demonstrating the advantage of employing chromatography before MS detection, the method developed by Schultz et al. measured more accurate concentrations of succinylacetone compared with a conventional flow injection analysis-MS/MS method. An (unidentified) interfering compound was chromatographically separated in Schultz et al.'s method, whereas in the conventional method, the interfering compound would incorrectly add to the perceived concentration of succinylacetone.

Nybo et al. compared vitamin D concentrations in DBS and plasma at different storage times and conditions, using a Phenomenex Gemini C18 column with a Thermo Scientific Discovery Ultra TSQ triple quadrupole for the DBS analysis [21]. The authors concluded that there was no significant difference between DBS and plasma vitamin D concentrations and that DBS is a suitable sample material for vitamin D measurement in multicenter studies, which can involve sampling at places with large temperature differences. Vitamin D appears to be a stable DBS analyte as freeze-thaw-freeze cycles did not lead to significant concentration changes.

A DBS LC-MS method for trimethylamine N-oxide and eight related compounds, all associated with cardiovascular diseases (e.g., stroke and heart failure), was developed and validated by Cho et al., using a SeQuant ZIC-HILIC column and a Thermo Scientific Q Exactive hybrid quadrupole–Orbitrap mass spectrometer [45]. To prevent volatilization of trimethylamine, an "on-spot reaction" method with pre-treatment of the DBS card with diluted HCl was successfully employed.

3.1.2 | Xenobiotics

In the USA, opioid overdoses represent a large public health threat, urging a need to monitor opioid exposure. For assessment of human exposure to fentanyl and fentanyl analogs, an LC-MS/MS method for fentanyl, twelve fentanyl analogs, and four metabolites (e.g., 3-methylfentanyl and ocfentanil) was developed and validated by Seymour et al., see Figure 4 and reference [46]. Using an Agilent Pursuit pentafluorophenyl column and a Sciex 6500 triple quadrupole MS, the authors demonstrated the importance of chromatography as 3-methylfentanyl and α-methylfentanyl have the same MRM transition, and without the chromatographic separation, it would have been impossible to determine each individual compound's concentration.

TDM of immunosuppressants using at-home sampled DBS could greatly reduce the number of hospital visits that patients with organ transplantation, chemotherapy, or autoimmune disorder have to endure. Using DBS-MS 500 from CAMAG, an automated DBS extraction system, coupled with LC-MS/MS instrumentation, Deprez and Stove developed and validated a method for quantification of four immunosuppressants (tacrolimus, sirolimus, everolimus, and cyclosporin A) [47]. Deprez and Stove employed a Phenomenex Kinetex Phenylhexyl column. Although the authors concluded that testing their method on real capillary DBS samples is needed to confirm the clinical applicability and that further examination of the Hct effect is necessary, the comparison of venous whole blood samples with venous DBS concentrations was satisfactory, demonstrating the potential of their developed method for TDM of immunosuppressants. Braal et al. used a Waters Acquity HSS T3 column with a Waters TQ (triple quadrupole) MS for quantification of the breast cancer drug ribociclib, demonstrating their DBS LC-MS/MS method to be suitable for ribociclib pharmacokinetic studies [48].

A DBS LC-MS/MS method for the identification and quantification of antidepressants and antipsychotics was developed by Moretti et al., employing a Phenomenex Kinetex C18 column [49]. In general, the agreement between DBS and corresponding conventional blood sample concentrations in real post-mortem cases was good, indicating that DBS is a suitable material for LC-MS/MS analysis of post-mortem samples.

Quantification of steroids in serum is used for the diagnosis of several endocrine disorders and for anti-doping tests. In these contexts, DBS sampling could be simpler than urine sampling and less invasive than venous blood sampling. Salamin et al. described a DBS LC-MS/MS method for quantification of eleven free and eight conjugated steroids, validated according to World-Anti Doping Agency requirements [50]. Using a Phenomenex Kinetex C18 column with a Waters Xevo-TQ S triple quadrupole, DBS (HemaXis DB10 kits) and serum from healthy women were analyzed, with the conclusion that the results were in high agreement. The authors also demonstrated stability of the investigated steroids for 1–3 weeks at room temperature, 4 and −20°C, indicating that DBS is a suitable sample material for anti-doping purposes as the samples can be transported at ambient temperatures, making sampling in the field easier.

3.1.3 | Global metabolomics

Recent research applying LC-MS to global metabolomics is given below, with examples of approaches for biomarker discovery and characterization of metabolites in various applications and areas. Both RP and HILIC approaches are used, and all examples utilize high-resolution mass spectrometers (Q Exactive Orbitrap and Q-TOF).

To assess the suitability of using DBS in autism spectrum disorder (ASD) research, Courraud et al. compared NBS samples of cases (i.e., newborns who were given an ASD diagnosis at a later time point) with controls [18]. Using RP chromatography (Waters Acquity UPLC BEH C18 column) with a Thermo Scientific Q Exactive Orbitrap, the analysis covered 31 chemical classes (with a great diversity, for example, amino acids, acylcarnitines, and steroids) and a large number of metabolites known to be associated with ASD, showing that this approach has a great potential in further ASD research. No obvious difference between cases and controls was detected, possibly due to the small sample size. However, several confounders were identified, such as gestational age and month of birth, which should be taken into consideration in metabolomics studies on NBS DBS.

As many facilities store DBS at 4–5°C, Yu et al. demonstrated the utility of refrigerated DBS (archived NBS samples, ∼4°C) stored for up to 21 years for profiling of metabolites and measurement of hemoglobin, the latter for Hct correction by spectroscopy [51]. With an Agilent 6545 Q-TOF MS, a ZIC-HILIC (zwitterionic stationary phase) column was employed for polar metabolites, while for semi- and non-polar metabolites, a Zorbax Eclipse Plus C18 RRHD column was used. In addition, Yu et al. propose normalization models to correct for and reduce the effect of variance introduced by, for example, Hct effects and storage time, improving the usefulness of archived DBS for metabolomics.

Skogvold et al. (Figure 5) developed and optimized a Pursuit XRs Diphenyl column LC-Q Exactive Orbitrap

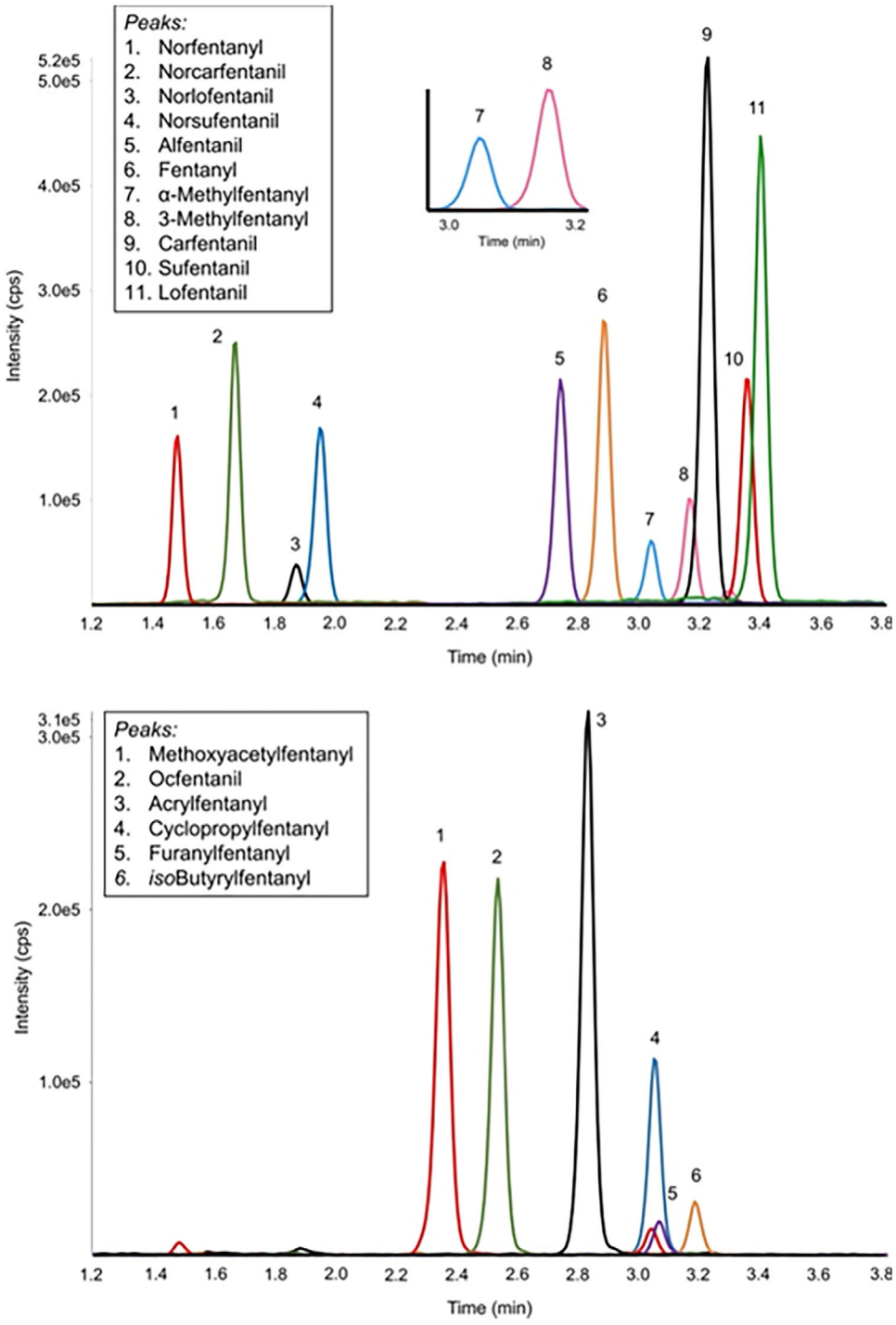


FIGURE 4 A LC-MS/MS method developed by Seymour et al. was demonstrated to cover several fentanyl analogs and metabolites, emphasizing the utility of chromatography with separation of compounds sharing the same multiple reaction monitoring (MRM) transition (3-methylfentanyl and α-methylfentanyl). Reprinted [46] (public domain).

MS metabolomics method for DBS demonstrating a large metabolome coverage with a polarity range of logP −4.4–8.8 of detected metabolites [17]. Several of the detected metabolites serve as biomarkers for diseases. The method was also demonstrated to be able to detect differences between nutritional states in DBS samples taken after free diet, overnight fasting, and prolonged fasting. With paired plasma and DBS samples, both HILIC (Waters UPLC BEH Amide column) and RP (with Waters UPLC BEH C18 columns) coupled with a Thermo Scientific Q Exactive Orbitrap was employed by Tobin et al. [15] to assess the use of DBS in metabolomics for biomarker discovery in

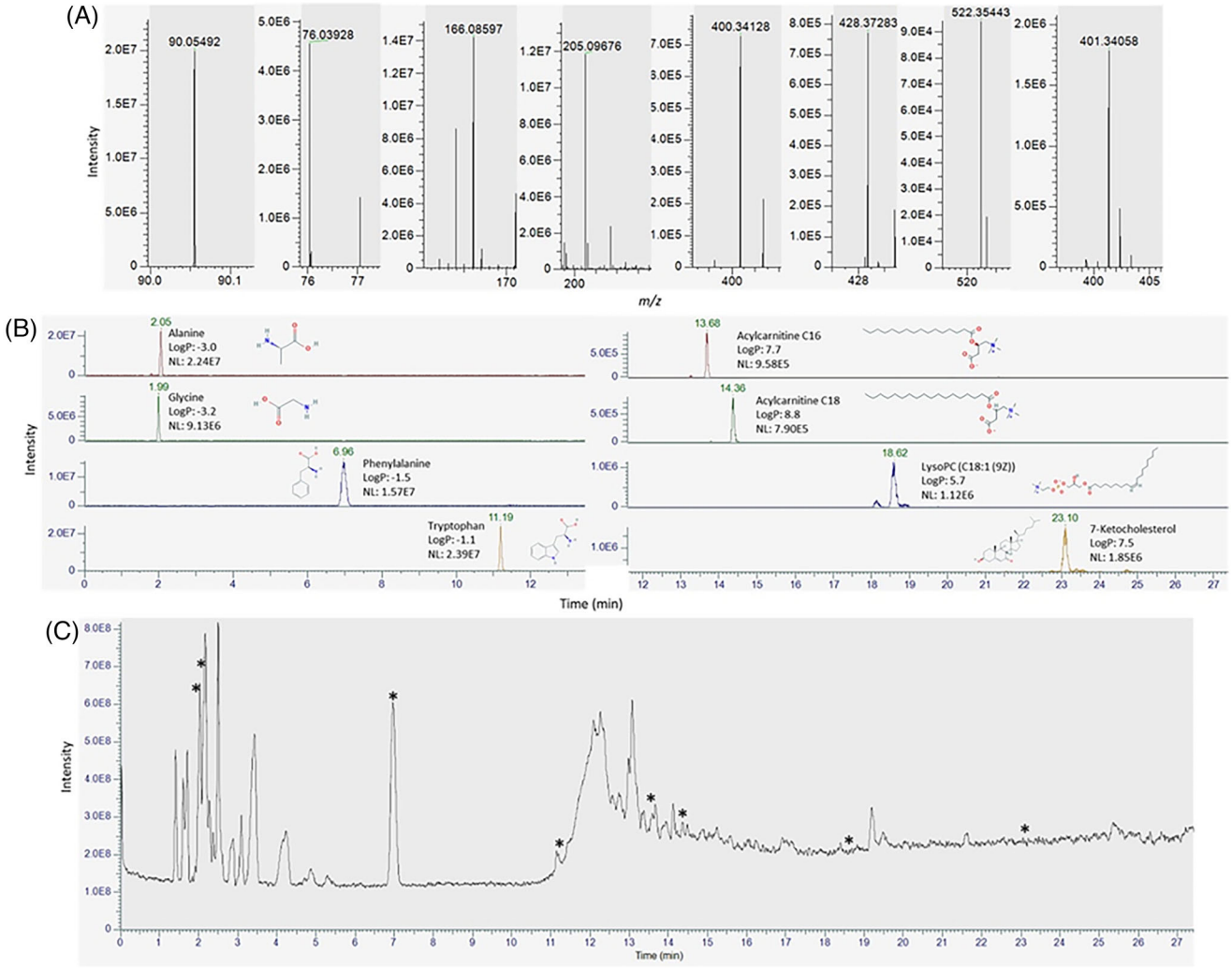


FIGURE 5 Illustration of the wide polarity coverage of endogenous metabolites detected by the global metabolomics LC-MS method developed by Skogvold et al. Reprinted from [17] with permission under the Creative Commons Attribute licensing.

a clinical trial of pregnant women living with HIV. The authors concluded that DBS is suitable for predictive modeling and discovery of biomarkers in this context, and that the choice of sample material should be built on the probability of detecting relevant biomarkers as well as practical reasons.

Rus et al. employed LC-MS (Kinetex EVO C18 column with a Waters IMS-Q-TOF MS) in a study of optimal conditions for the search for biomarkers using metabolomics, especially for lysosomal storage diseases in DBS sampled from both children and adults [52]. In addition, the effect of storage time- and conditions and types of extraction solvents were assessed. The authors concluded that DBS metabolites are largely affected by storage time- and conditions, and that using methanol as extraction solvent is recommended to increase the chance of discovering biomarkers.

3.2 | Large molecules: proteins and peptides

Determination of proteins from DBS in a clinical setting has been performed since the early seventies. Thielmann and Moreira Aquino developed a screening test for sickle cell anemia based on the electrophoretic distinction between hemoglobin A (Hb A) and hemoglobin S. The latter is a marker for sickle cell anemia [53]. They used dried blood instead of capillary blood to avoid the necessity of refrigerating to stabilize the proteins. This prevented degradation from samples taken in and transported from the Mediterranean and tropical areas. In 1986, the first report on MS for determining proteins in a clinical setting from DBS samples was published. Wada et al. screened 80,000 neonates to study the prevalence of Hb F Yamaguchi ($^{A}\gamma^{T}$ 80 Asp→Asn) and other Hb variants

[54]. At first instance, all samples were screened using isoelectric focusing in polyacrylamide slab gels. The abnormal variants from this screening were subjected to tryptic digestion followed by single MS analysis (i.e., bottom-up proteomics) to identify masses of abnormal peptides. Confirming amino acid sequencing was carried out using gas-phase sequencing of the abnormal peptides. Using this strategy, the authors were able to distinguish between structural Hb-variants.

After these pioneering studies, several papers have been published with a focus on protein determination from blood in the dried state using LC-MS. With the focus on the recent literature, mainly four categories of protein analysis from dried blood emerge: targeted protein determination of endogenous proteins (biomarkers), targeted protein determination of protein drugs (TDM and doping analysis), global proteomics analysis, as well as innovative- and emerging techniques.

3.2.1 | Targeted determination of endogenous proteins/biomarker monitoring

From an analytical perspective, it is logical that these abovementioned pioneering studies were performed on Hb since this is the most abundant protein present in whole blood: sample clean-up to avoid matrix effects is hardly necessary nor enrichment to be able to reach low detection limits. To date, there has been a significant effort in the mass spectrometric determination of Hb variants, and advances in the field have been thoroughly reviewed by Dasauni et al. [55]. In two recent studies, the advantages of high-resolution MS (HRMS) were shown in top-down and bottom-up approaches with and without chromatography. A comparative study between a top-down HPLC-UV analysis and a combination of top-down and bottom-up flow injection HRMS analysis was carried out on more than 5500 de-identified neonatal DBS samples. The conclusion was drawn that HRMS without any chromatographic separation was a fast, cheap, and highly specific way to differentiate between many Hb variants [24]. Also the use of DIA in nano-LC HRMS made differentiation between different Hb variants possible in dried blood from volumetric absorptive microsampling [56].

Proteins with a lower abundance have also been determined from DBS. The most common is the bottom-up approach, combined with the use of antibodies for clean-up and enrichment of the analyte of interest. This can be done by antibody-based affinity extraction before tryptic digestion (anti-protein antibodies) [57], or after tryptic digestion (using anti-peptide antibodies -SISCAPA) [58, 59]. To obtain good detection limits, mainly selected reaction monitoring on triple quadrupole mass spectrometers coupled to chromatographic separation is used [57, 58].

3.2.2 | Targeted determination of exogenous proteins/TDM and doping analysis

Besides the determination of endogenous proteins for diagnostic purposes, the importance of the determination of exogenous proteins is increasing.

Chiu et al. developed a method to extract four different monoclonal antibodies (mAbs) from 15 µl dried blood: bevacizumab, trastuzumab, nivolumab and tocilizumab were extracted using protein G beads followed by a tryptic digest and signature peptide determination in the MRM mode on a UHPLC equipped with a triple quadrupole MS [25]. Its applicability for TDM was tested in a small group ($n = 6$) of patients. Another clinically relevant application is performance of pharmacokinetic measurements [60].

There have also been increasing efforts to determine the presence and concentration of illicit drugs in DBS. Lange et al. developed and validated two complementary LC–HRMS methods for analyzing sotatercept in DBS for doping controls. Sotatercept was selectively extracted from DBS using protein G coupled beads and after a bottom-up approach determined with LC-HRMS [61], showing the proof-of-concept for the determination of this type of drugs in DBS. Also, top-down approaches are feasible in doping analysis: Thomas and Thevis report a strategy facilitating the analysis of insulin and its synthetic or animal analogs (human, Lispro, Aspart, Glulisine, Glargine, Detemir, Tresiba, and porcine and bovine insulin) from DBS [62]. All these efforts have, among others, lead to an increased attention of the World Anti-Doping Association and since 2021 the use of DBS in sports is approved [63].

3.2.3 | Global proteomics

Chambers et al. were the first to carry out a global proteomic experiment on DBS [64]. Their bottom-up approach allowed them to qualify 253 proteins from the dried blood compared to 223 from un-depleted whole blood. This revealed that, for global proteomic experiments, DBS was at least as good as whole blood.

More recent efforts with MALDI-ToF allow proteomic profiling of neonatal DBS samples which have been stored for up to 2 years at ambient conditions. With a 72 h extraction from paper followed by conventional reduction, alkylation, and tryptic digestion they were able to identify 30 proteins [65]. Although this is a much lower number than feasible, it needs to be stressed that no chromatogra-

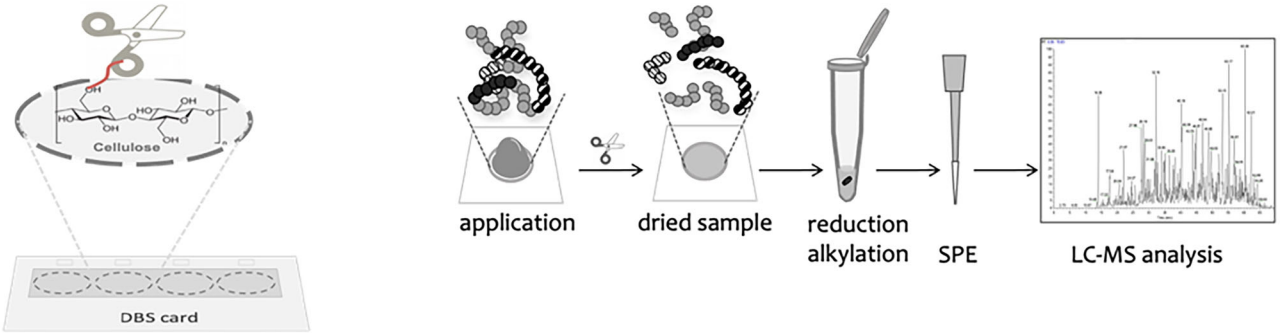


FIGURE 6 Trypsin covalently bound to cellulose (as shown on the left of the figure) allows proteins to be digested from the moment the sample is applied to the dried blood spot (DBS) card.

phy was carried out before the MS analysis. The effect of chromatographic separation on the protein determination from dried samples was shown by Wölter et al. [66]. In their study, dried serum samples (from Noviplex cards) were analyzed using MALDI-ToF and nano-LC-MS after a bottom-up approach. The MALDI-ToF was able to detect 8 proteins while the nano-LC-MS allowed 72 proteins to be detected, both from the same sample. In this case chromatography circumvents possible ion-suppression and possible limitations caused by a narrow dynamic range, thus increasing the identification rate.

Although the above-mentioned studies show the feasibility of performing global proteomic experiments from dried matrix samples, the huge potential is shown by Nakajima et al. [26]. Their protocol involves a combination of sodium carbonate precipitation and sonication of paper in presence of zirconia beads followed by trypsination. Analysis was performed using nano-LC-HRMS which was operated in the DIA mode. With this approach they were able to identify almost 2000 proteins clearly showing that with the right protocol, global proteomics from DBS reveals a huge amount of information.

3.2.4 | Emerging variants

As mentioned above, the determination of proteins from DBS is a challenging task, especially when low detection limits need to be achieved. Most of the bottom-up approaches are based on the use of trypsin since this proteolytic enzyme produces MS-friendly doubly charged peptides. Additionally, affinity-based clean-up steps are required to remove highly abundant proteins. Such clean-up steps overcome limitations caused by the dynamic range of the MS as well as reduce suppression from the matrix.

Efforts have been made to integrate preparation steps either into the workflow or into the DBS paper itself. Skjærvø et al. showed in 2017 and 2018 that it is possible to start the digestion of proteins at the moment the sample is applied to filter paper: both with non-covalently [67], and covalently bound trypsin (Figure 6) [68]. Although successful, a major drawback with covalent binding via 2-hydroxyethyl methacrylate-co-2-vinyl-4,4-dimethyl azlactone chemistry was that the paper lost much of its absorbing properties [68]. This was overcome by covalent binding of trypsin via periodate oxidation [69, 70]. In all cases, the integrated proteolysis step allowed to digest even complex samples as serum and whole blood identifying up to 200 proteins per spot. Additionally, it appeared that paper with covalently bound trypsin was surprisingly stable: it did not lose its activity when kept dry for 4 months [70].

Not only the proteolysis sample preparation step was integrated, but also mAbs were covalently bound to filter paper, making affinity-based sample clean-up possible. 2-Hydroxyethyl methacrylate-co-2-vinyl-4,4-dimethyl azlactone modification of cellulose was used in the case of direct immobilization of mAbs to paper [71, 72]. For the model protein human chorionic gonadotropin detection limits down to 1 ng/ml in 20 μl dried human serum were obtained.

In addition to covalently bound mAbs carrying out affinity-based sample clean-up, cellulose with covalently bound streptavidin has its potential: streptavidin strongly binds biotinylated mAbs which in turn can perform affinity-based sample clean-up (Figure 7). This more versatile approach can make tailor-made production of smart affinity samplers less challenging [73].

4 | CONCLUDING REMARKS

DBS-based analysis has regained a well-deserved popularity due to its unleashed potential in clinical utility. For one, DBS allows cheap and simple sampling and ambient temperature transport and storage, thus providing access to laboratory services from any geographical outskirt, and the privacy of the patient's own home. Secondly, the

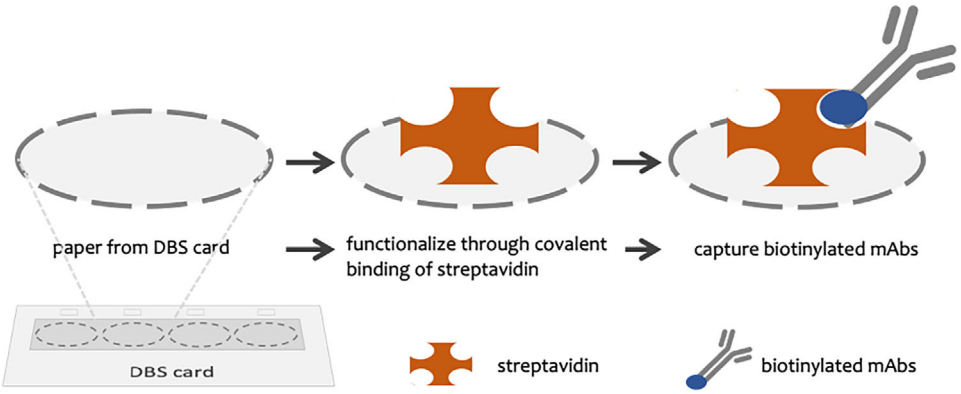


FIGURE 7 Covalently bound streptavidin can act as an anchor for biotinylated antibodies.

advent of MS-based targeted analyses, proteomics, and metabolomics has opened a new world of opportunities for new biomarker discovery and the simultaneous detection of a multitude of analytes facilitating NBS, advanced diagnostics, TDM, and other forms of therapeutic monitoring as well as providing new insights into physiological responses to diet, exercise, and environmental and other exposures. Altogether, DBS- and MS-based analyses has the potential to reduce the unacceptable global health inequality with respect to laboratory diagnostics and treatment, as well as provide the full spectrum of biochemical omics-based information into the forefront of advanced medicine.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Léon Reubsaet https://orcid.org/0000-0001-5165-2701
Steven Ray Wilson https://orcid.org/0000-0002-9755-1188

REFERENCES

1. Rajadhyaksha M, Londhe V. Microsampling: A role to play in Covid-19 diagnosis, surveillance, treatment and clinical trials. Drug Test Anal. 2021;13:1238–48.
2. Gates B. Dagens Medisin. Isachsen, H. B. 2023.
3. Bang IC, Blutzucker D. Wiesbaden, Germany 1913.
4. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics 1963;32:338–43.
5. Guthrie R. The origin of newborn screening. Screen. 1992;1:5–15.
6. Therrell BL, Padilla CD, Loeber JG, Kneisser I, Saadallah A, Borrajo GJC, et al. Current status of newborn screening worldwide: 2015. Semin Perinatol. 2015;39:171–87.
7. Mee JML, Korth J, Halpern B. Rapid and quantitative blood analysis for free fatty acids by chemical ionization mass spectrometry. Anal Lett. 1976;9:1075–83.
8. Grüner N, Stambouli O, Ross RS. Dried blood spots - preparing and processing for use in immunoassays and in molecular techniques. JoVE 2015;e52619.
9. 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.
10. Lim MD. Dried blood spots for global health diagnostics and surveillance: opportunities and challenges. Am J Trop Med Hyg. 2018;99:256–65.
11. Moat SJ, George RS, Carling RS. Use of dried blood spot specimens to monitor patients with inherited metabolic disorders. Int J Neonatal Screening. 2020;6:26.
12. Ververi C, Vincenti M, Salomone A. Recent advances in the detection of drugs of abuse by dried blood spots. Biomed Chromatogr. 2022;e5555.
13. Sadones N, Capiau S, Kesel PMD, Lambert WE, Stove CP. Spot them in the spot: analysis of abused substances using dried blood spots. Bioanalysis 2014;6:2211–27.
14. Le J, Peng R, Yang S-L, Chen J-L, Cheng J-R, Li Y, et al. Quantification of immunosuppressants from one 3.2 mm dried blood spot by a novel cold-induced phase separation based LC-MS/MS method. Anal Chim Acta. 2022;1210:339889.
15. Tobin NH, Murphy A, Li F, Brummel SS, Taha TE, Saidi F, et al. Comparison of dried blood spot and plasma sampling for untargeted metabolomics. Metabolomics 2021;17:62.
16. Dubland JA, Rakić B, Vallance H, Sinclair G. Analysis of 2-methylcitric acid, methylmalonic acid, and total homocysteine in dried blood spots by LC-MS/MS for application in the newborn screening laboratory: A dual derivatization approach. J Mass Spectrom Adv Clin Lab. 2021;20:1–10.
17. Skogvold HB, Sandås EM, Østeby A, Løkken C, Rootwelt H, Rønning PO, et al. Bridging the polar and hydrophobic metabolome in single-run untargeted liquid chromatography-mass spectrometry dried blood spot metabolomics for clinical purposes. J Proteome Res. 2021;20:4010–21.
18. Courraud J, Ernst M, Svane Laursen S, Hougaard DM, Cohen AS. Studying autism using untargeted metabolomics in newborn screening samples. J Mol Neurosci. 2021;71:1378–93.
19. Huang X, Liu X, Wang Y, Zhang J. Determination of polymyxin B in dried blood spots using LC-MS/MS for therapeutic drug monitoring. J Chromatogr B. 2022;1192:123131.
20. Zhan X, Han L, Qiu W, Gu X, Guo J, Chang S, et al. Steroid profile in dried blood spots by liquid chromatography tandem mass

spectrometry: Application to newborn screening for congenital adrenal hyperplasia in China. Steroids 2022;185:109056.
21. Nybo M, Fruekilde PN, Andersen-Ranberg K. Measurement of vitamin D in dried blood spots stored under different temperature conditions. Ann Clin Biochem. 2021;58:461–7.
22. Möller I, Held K, Klimpel D, Nadulski T, Dufaux B. Development and validation of an LC–MS/MS method for relevant drugs in epilepsy patients using dried blood spots. Biomed Chromatogr. 2021;35:e5130.
23. Eibak LEE, Hegge AB, Rasmussen KE, Pedersen-Bjergaard S, Gjelstad A. Alginate and chitosan foam combined with electromembrane extraction for dried blood spot analysis. Anal Chem. 2012;84:8783–9.
24. Wiesinger T, Mechtler T, Schwarz M, Xie X, Grosse R, Cobos PN, et al. Investigating the suitability of high-resolution mass spectrometry for newborn screening: identification of hemoglobinopathies and β-thalassemias in dried blood spots. Clin Chem Lab Med. 2020;58:810–6.
25. Chiu H-H, Tsai Y-J, Lo C, Liao H-W, Lin C-H, Tang S-C, et al. Development of an LC-MS/MS method to simultaneously quantify therapeutic mAbs and estimate hematocrit values in dried blood spot samples. Anal Chim Acta. 2022;1189:339231.
26. Nakajima D, Kawashima Y, Shibata H, Yasumi T, Isa M, Izawa K, et al. Simple and sensitive analysis for dried blood spot proteins by sodium carbonate precipitation for clinical proteomics. J Proteome Res. 2020;19:2821–7.
27. Velghe S, Delahaye L, Stove CP. Is the hematocrit still an issue in quantitative dried blood spot analysis? J Pharm Biomed Anal. 2019;163:188–96.
28. Barr DB, Kannan K, Cui Y, Merrill L, Petrick LM, Meeker JD, et al. The use of dried blood spots for characterizing children's exposure to organic environmental chemicals. Environ Res. 2021;195:110796.
29. Baillargeon KR, Brooks JC, Miljanic PR, Mace CR. Patterned dried blood spot cards for the improved sampling of whole blood. ACS Measurement Sci Au. 2022;2:31–8.
30. Haijes HA, Willemsen M, Van der Ham M, Gerrits J, Pras-Raves ML, Prinsen H, et al. Direct infusion based metabolomics identifies metabolic disease in patients' dried blood spots and plasma. Metabolites 2019;9:12.
31. Dénes J, Szabó E, Robinette SL, Szatmári I, Szőnyi L, Kreuder JG, et al. Metabonomics of newborn screening dried blood spot samples: a novel approach in the screening and diagnostics of inborn errors of metabolism. Anal Chem. 2012;84:10113–20.
32. Vreeker GCM, Bladergroen MR, Nicolardi S, Mesker WE, Tollenaar R, van der Burgt YEM, et al. Dried blood spot N-glycome analysis by MALDI mass spectrometry. Talanta 2019;205: 120104.
33. Uribe B, González O, Blanco ME, Albóniga OE, Alonso ML, Alonso RM. Analysis of the heterogeneous distribution of amiloride and propranolol in dried blood spot by UHPLC-FLD and MALDI-IMS. Molecules 2019;24:4320.
34. Soares S, Castro T, Rosado T, Fernández N, Barroso M, Gallardo E. New analytical approach to determine organophosphorus insecticides in blood by dried matrix spots sampling and GC-MS/MS. Anal Bioanal Chem. 2018;410:7955–64.
35. Min KL, Ryu JY, Chang MJ. Development and clinical applications of the dried blood spot method for therapeutic drug monitoring of anti-epileptic drugs. Basic Clin Pharmacol Toxicol. 2019;125:215–36.
36. Jobst KJ, Arora A, Pollitt KG, Sled JG. Dried blood spots for the identification of bio-accumulating organic compounds: current challenges and future perspectives. Curr Opin Environ Sci Health. 2020;15:66–73.
37. DiBattista A, McIntosh N, Lamoureux M, Al-Dirbashi OY, Chakraborty P, Britz-McKibbin P. Temporal signal pattern recognition in mass spectrometry: A method for rapid identification and accurate quantification of biomarkers for inborn errors of metabolism with quality assurance. Anal Chem. 2017;89:8112–21.
38. Ryšavá L, Dvořák M, Kubáň P. Dried blood spot self-sampling with automated capillary electrophoresis processing for clinical analysis. Angew Chem Int Ed. 2021;60:6068–75.
39. Le Faouder P, Soullier J, Tremblay-Franco M, Tournadre A, Martin J-F, Guitton Y, et al. Untargeted lipidomic profiling of dry blood spots using SFC-HRMS. Metabolites 2021;11:305.
40. Jain A, Morris M, Lin EZ, Khan SA, Ma X, Deziel NC, et al. Hemoglobin normalization outperforms other methods for standardizing dried blood spot metabolomics: A comparative study. Sci Total Environ. 2023;854:158716.
41. Walsby-Tickle J, Gannon J, Hvinden I, Bardella C, Abboud MI, Nazeer A, et al. Anion-exchange chromatography mass spectrometry provides extensive coverage of primary metabolic pathways revealing altered metabolism in IDH1 mutant cells. Commun Biol. 2020;3:247.
42. Verplaetse R, Henion J. Quantitative determination of opioids in whole blood using fully automated dried blood spot desorption coupled to on-line SPE-LC-MS/MS. Drug Test Anal. 2016;8:30–8.
43. Kilgore MB, Platis D, Lim T, Isenberg S, Pickens CA, Cuthbert C, et al. Development of a universal second-tier newborn screening LC–MS/MS method for amino acids, lysophosphatidylcholines, and organic acids. Anal Chem. 2023;95:3187–94.
44. Schultz MJ, Netzel BC, Singh RH, Pino GB, Gavrilov DK, Oglesbee D, et al. Laboratory monitoring of patients with hereditary tyrosinemia type I. Mol Genet Metab. 2020;130:247–54.
45. Cho H-D, Kim J, Lee J-Y, Kim Y-Y, Lee Y, Jo E, et al. A novel dried blood spots analysis combined with on-spot reaction for determination of trimethylamine N-oxide and its related compounds. Talanta 2020;210:120639.
46. Seymour C, Shaner RL, Feyereisen MC, Wharton RE, Kaplan P, Hamelin EI, et al. Determination of fentanyl analog exposure using dried blood spots with LC–MS-MS. J Anal Toxicol. 2018;43:266–76.
47. Deprez S, Stove CP. Fully automated dried blood spot extraction coupled to liquid chromatography-tandem mass spectrometry for therapeutic drug monitoring of immunosuppressants. J Chromatogr A. 2021;1653:462430.
48. Braal CL, Lam MH, Rienks T, van Tilborg CJ, Heuts W, Heijns JB, et al. Quantification of ribociclib in dried blood spots by LC–MS/MS: Method development and clinical validation. J Pharm Biomed Anal. 2021;201:114118.
49. Moretti M, Freni F, Valentini B, Vignali C, Groppi A, Visonà SD, et al. Determination of antidepressants and antipsychotics in dried blood spots (DBSs) collected from post-mortem samples and evaluation of the stability over a three-month period. Molecules 2019;24:3636.

50. Salamin O, Nicoli R, Xu C, Boccard J, Rudaz S, Pitteloud N, et al. Steroid profiling by UHPLC-MS/MS in dried blood spots collected from healthy women with and without testosterone gel administration. J Pharm Biomed Anal. 2021;204:114280.
51. Yu M, Dolios G, Yong-Gonzalez V, Björkqvist O, Colicino E, Halfvarson J, et al. Untargeted metabolomics profiling and hemoglobin normalization for archived newborn dried blood spots from a refrigerated biorepository. J Pharm Biomed Anal. 2020;191:113574.
52. 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.
53. Thielmann K, Aquino AM. Whole blood samples dried and stored on filter paper as substrate for the electrophoretic separation on hemoglobin S from hemoglobin A. A screening procedure. Clin Chim Acta. 1971;35:237–8.
54. Wada Y, Fujita T, Kidoguchi K, Hayashi A. Fetal hemoglobin variants in 80,000 Japanese neonates: high prevalence of Hb F Yamaguchi (AγT 80 Asp→Asn). Hum Genet. 1986;72:196–202.
55. Dasauni P, Chhabra V, Kumar G, Kundu S. Advances in mass spectrometric methods for detection of hemoglobin disorders. Anal Biochem. 2021;629:114314.
56. Lima DA, Schuch RA, Salgueiro JS, Pintão MCT, Carvalho VM. Evaluation of volumetric absorptive microsampling and mass spectrometry data-independent acquisition of hemoglobin-related clinical markers. J Proteome Res. 2022;21:1816–28.
57. Collins CJ, Yi F, Dayuha R, Duong P, Horslen S, Camarata M, et al. Direct measurement of ATP7B peptides is highly effective in the diagnosis of Wilson disease. Gastroenterology 2021;160:2367–82.e1.e2361.
58. Anderson L, Razavi M, Pope ME, Yip R, Cameron L, Bassini-Cameron A, et al. Precision multiparameter tracking of inflammation on timescales of hours to years using serial dried blood spots. Bioanalysis 2020;12:937–55.
59. Hsiao Y-C, Lin S-Y, Chien K-Y, Chen S-F, Wu C-C, Chang Y-T, et al. An immuno-MALDI mass spectrometry assay for the oral cancer biomarker, matrix metalloproteinase-1, in dried saliva spot samples. Anal Chim Acta. 2020;1100:118–30.
60. Gao X, Chen C, Geng D, Bateman KP, Shi S, Woolf EJ, et al. Volumetric absorptive microsampling (VAMS®) in therapeutic protein quantification by LC-MS/MS: Investigation of anticoagulant impact on assay performance and recommendations for best practices in method development. J Pharm Biomed Anal. 2021;196:113895.
61. Lange T, Walpurgis K, Thomas A, Geyer H, Thevis M. Development of two complementary LC–HRMS methods for analyzing sotatercept in dried blood spots for doping controls. Bioanalysis 2019;11:923–40.
62. Thomas A, Thevis M. Analysis of insulin and insulin analogs from dried blood spots by means of liquid chromatography–high resolution mass spectrometry. Drug Test Anal. 2018;10:1761–8.
63. https://www.wada-ama.org/en/news/wada-executive-committee-approves-technical-document-innovative-dried-blood-spot-testing Accessed March 24, 2023
64. Chambers AG, Percy AJ, Hardie DB, Borchers CH. Comparison of proteins in whole blood and dried blood spot samples by LC/MS/MS. J Am Soc Mass Spectrom. 2013;24:1338–45.
65. Samenuk GM, Kelley AR, Perry G, Bach SBH. Rapid method towards proteomic analysis of dried blood spots by MALDI mass spectrometry. Clin Mass Spectrom. 2019;12:30–6.
66. Wölter M, Russ M, Okai CA, Rath W, Pecks U, Glocker MO. Comparison of blood serum protein analysis by MALDI-MS from either conventional frozen samples or storage disc-deposited samples: A study with human serum from pregnant donors and from patients with intrauterine growth restriction. Eur J Mass Spectrom. 2019;25:381–90.
67. Skjærvø Ø, Rosting C, Halvorsen TG, Reubsaet L. Instant on-paper protein digestion during blood spot sampling. Analyst 2017;142:3837–47.
68. Ø S, Halvorsen TG, Reubsaet L. Smart blood spots for whole blood protein analysis. Analyst 2018;143:3184–90.
69. Pizzi E, Halvorsen TG, Koehler CJ, Reubsaet L. Next-generation dried blood spot samplers for protein analysis: describing trypsin-modified smart sampling paper. Separations 2021;8:66.
70. Nguyen MT, Halvorsen TG, Thiede B, Reubsaet L. Smart proteolysis samplers for pre-lab bottom-up protein analysis – Performance of on-paper digestion compared to conventional digestion. Sep Sci Plus. 2022;5:171–83.
71. Skjærvø Ø, Solbakk EJ, Halvorsen TG, Reubsaet L. Paper-based immunocapture for targeted protein analysis. Talanta 2019;195:764–70.
72. Skjærvø Ø, Halvorsen TG, Reubsaet L. All-in-one paper-based sampling chip for targeted protein analysis. Anal Chim Acta. 2019;1089:56–65.
73. Johannsen C, Haq AU, Reubsaet L, Halvorsen TG. On the spot immunocapture in targeted biomarker analysis using paper-bound streptavidin as anchor for biotinylated antibodies. Anal Bioanal Chem. 2022;414:5979–89.

How to cite this article: Skogvold HB, Rootwelt H, Reubsaet L, Elgstøen KBP, Wilson SR. Dried blood spot analysis with liquid chromatography and mass spectrometry: Trends in clinical chemistry. J Sep Sci. 2023;46:2300210. https://doi.org/10.1002/jssc.202300210