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A REVIEW :THE USE OF DRIED SALIVA SPOTS (DSS) FOR THE POSSIBLE MONITORING OF TREATMENT DRUGS AND THE DIAGNOSIS OF DISEASE

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ABSTRACT

Scientific researchers' interest in noninvasive sample techniques for illness diagnosis and therapeutic medication monitoring has grown over the past few years. Dried saliva spot (DSS) was employed as a saliva collection procedure for the first time. Using an organic solvent, 50 µL of saliva were extracted using the DSS process after being collected on filter paper. Liquid chromatography and mass spectrometry were employed in the DSS to identify the local anaesthetic lidocaine as a model substance. This has led to the widespread use of dry saliva spot (DSS), a sampling method for gathering dried saliva samples, as an alternate matrix to serum for the detection of target molecules. The effectiveness of the preparation and analysis of biological samples is increased when the DSS method is used in conjunction with a highly sensitive detection instrument. More topics related to the DSS method, such as dried blood spots, dried plasma spots, and dried matrix spots, are covered. Saliva offers a benefit over other biological fluids used in dried spot procedures, such as serum, tears, urine, and plasma, in terms of sample collection from young children or people with disabilities. By demonstrating several dried spot methods, this study seeks to offer essential strategies and principles for dried spot methods to investigate biological materials. In this article, we review current developments in DSS techniques from June 2014 to March 2021 and talk about the benefits and drawbacks of the main components of this technique, such as sample preparation and method validation. Finally, we discuss the difficulties and potential of these techniques in real-world settings.

Keywords: Human saliva, Dried saliva spot, Therapeutic drug monitoring, Disease diagnosis

1. Introduction

The most often utilised biological fluids for the detection of metabolites are typically blood (including plasma, serum, or whole blood), urine, and saliva. This is important for therapeutic drug monitoring (TDM) and illness diagnosis [1]. However, standard biological fluids used in TDM and illness detection frequently require samples in large quantities that are complex and potentially unstable, making it challenging to store and transfer the samples [2,3]. Human saliva may contain drugs and metabolites.Children and elderly individuals can benefit from saliva sampling because it is quicker and less invasive than plasma. Compared to plasma or blood, saliva offers special benefits such simplicity of sampling, management, and transport. Due to its benefits, such as decreased tissue damage, reduced sampling volume, and convenience, the usage of dried spot sampling techniques has grown over the past few decades, opening up opportunities for technical advancement in a number of sectors [4].

Dried blood spot (DBS), dried matrix spot (DMS), dried saliva spot (DSS), and other dried biological fluid spot technologies are employed more frequently in fundamental medical research than traditional sample methods.

DBS technology is an established method whose application is growing. Over the past ten years, DMS and DSS have progressed quickly on the basis of the DBS approach. The non-blood matrix is analysed using the DMS technique. To improve the visibility of transparent fluids spotted onto DMS cards, this technique frequently makes use of filter paper with a colour indicator. Saliva collection is painless and non-invasive. Therefore, saliva is more effective as a diagnostic fluid than blood and other non-blood matrices (cerebrospinal fluid, synovial fluid, tears, urine, and plasma) [5–9]. Additionally, the DSS method typically uses 3-100 mL of saliva as the sampling volume, which facilitates quickly collecting a large number of samples. In terms of TDM and illness diagnosis, the DSS approach has achieved significant advancements.

This study provides a summary of the DSS technique's research activities from June 2014 to March 2021. This review focuses on the drawbacks of the DSS technique in terms of sample preparation and methodological verification, including the choice of suitable filter paper types and filter paper cutting equipment, internal standard addition, extraction settings, analyte stability, and instrument sensitivity. Additionally, information about DBS, dried urine spot (DUS), and DMS is provided. This review provides new procedures and approaches for the collecting, storage, and transportation of biological samples in addition to summarising the dry spot sampling techniques already in use. In the future, this technique can be applied to additional analytical sampling and analysis sectors in addition to the examination of biological samples.

2. DSS technique

Saliva samples can be collected using the well-known DSS sampling procedure by spotting salivary specimens on filter paper. Saliva sample collection is inherently non-invasive and painless; in addition, it doesn't require special training, can be done at home, poses virtually no infection risk, is well-tolerated by patients, and has a significant advantage when it comes to collecting samples from particular populations, such as children, the disabled, or people with anxiety disorders [10]. The parotid, sublingual, submandibular, and countless smaller salivary glands all generate saliva. Diverse salivary glands secrete saliva with different chemical compositions, as has been seen [6]. However, due to the extensive training needed for the specialist collecting the material, saliva from various salivary glands is not frequently employed. Whole saliva is simple to obtain and appropriate for use by participants and patients in scientific studies. Whether salivary production is basal or triggered affects the saliva's chemical composition as well. As a result, differences in salivary composition may result from different salivary collection techniques [9]. For example, passive drooling and direct saliva collection into a sampling tube are two methods for gathering entire, unstimulated saliva. The direct collection of saliva has the obvious drawback of potentially including more bacteria, which could interfere with the analysis of various chemicals. To gather stimulated entire saliva, several techniques such as gentle mastication, the application of citric acid, chewing on a cotton roll, and polystyrene foam swabs are typically utilised. These techniques have their own drawbacks; for instance, citric acid influences the analysis of testosterone. Because of this, researchers should select a sampling strategy that is best for analyte quantification. Saliva drips from the lower lip are gathered into the sampling tube for DSS sampling. This method of collecting saliva is appropriate since it is practical, does not call for additional sampling tools, and is appropriate for everyone. Brushing, eating, drinking, and chewing gum should be prohibited for at least 30 minutes prior to collection (save for water) [8].

The diameters and levels of liquid absorption of various filter sheets vary. The required amount of saliva ranges from 3-100 mL depending on the filter material that is chosen. After collecting the DSS samples, the salivary collection card is allowed to dry and is stored at room temperature. This property enhances saliva sample stability and preservation because saliva and target analytes stick to the filter paper. As a result, the benefit of DSS greatly lowers transportation expenses. The DSS is then extracted with an appropriate solvent using a combination of vortex- and ultrasound-assisted extraction (VAE) (UAE). A mobile phase or extraction solvent is then used to concentrate and reconstitute the extraction.

The diagnosis of numerous disorders [11–15], the bio-detection of numerous medications [16–4], and metabolomics analysis [25–26] have all benefited greatly from the widespread usage of DSS to date. The results of seven years' worth of literature research on DSS as a sampling technology (June 2014–March 2021) are presented . We pay special attention to the development of the DSS methods, which includes sample preparation and technique validation.

2.1 Different types of filter paper used in the DSS method

Four different types of filter paper are utilised in DSS among the known research that use it (Fig. 1). The dried saliva spots are difficult to see on the Whatman FTATM DMPK-C card (Fig. 1A), which was used to collect saliva samples. In order to enable the analyst to visually confirm the location of the dried sample spot, color-indicating cards (Fig. 1B) were created [27]. We advise comparing analyte recovery when saliva samples are collected using filter paper with a colour indicator to that obtained using a filter paper without a colour indicator because the colour additive can cause ionisation suppression.

Whatman FTATM DMPK cards come in two different varieties, FTA DMPK-A and FTA DMPK-B, and are typically used for DBS sample collection. Chemical reagents that can denaturize proteins are included on the FTA DMPK-A and FTA DMPK-B cards but not on the Whatman FTA DMPK-C card [28]. FTA DMPK cards were used in the DBS method to examine the extraction effectiveness and stability of plasma samples [29]. However, no information has been provided regarding the effectiveness and stability of saliva extraction from filter paper using the DSS approach. Therefore, future improvements to the DSS method should concentrate on the filter paper's performance and ensure that it is appropriate for the target analyte.

For the purpose of measuring lidocaine, regular filter paper with a predetermined diameter is utilised (Fig. 1C) [18]. The centre of the salivary spots, which are visually indicated places for biological sample collection, are punched. Analysts struggle to regulate where applied saliva samples are placed, though. This DSS type filter paper can be used to collect saliva samples, but the size of the samples themselves may be larger than the filter paper's fixed diameter collection places. In order to find uric acid in human saliva, a standard filter paper (Fig. 1D) is utilised [26]. This filter paper is first trimmed to the right size to stop the spit from spreading outside of the permitted area. The analysis can be more accurate if the position of the dried sample is accurately known because DSS spotted on filter paper is less evident than DBS. Utilizing ultraviolet (UV) light is one method to improve the visibility of dried saliva spots for DSS [23]. Utilizing a UV lamp for viewing has the benefit of not affecting the analyte recovery rate.

The DSS method often makes use of the WhatmanTM 903 protein saver card, commonly known as the Whatman 903 card (Fig. 1E). This filter paper is employed to safeguard proteins from biological materials, as its name suggests. The FTA Classic Card, which is impregnated with chemicals that lyse cells, denature proteins, and shield nucleic acids from nucleases, oxidative damage, and UV damage, is not the same as the WhatmanTM 903 Protein Saver Card. It has been demonstrated that this filter paper can find nucleic acids in blood [30–32]. A new technique for diagnosing oral squamous cell carcinoma was recently described by Hsiao et al. [11] using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MS). Streptococcus pneumoniae is stable in DSS stored with a desiccant for up to 1 month over a wide range of temperatures, and Krone et al. [13] described a reliable method using polymerase chain reaction in conjunction with the WhatmanTM 903 protein saver card for the detection of Streptococcus pneumoniae carriage in human saliva. As a result, this filter paper is also appropriate for examining salivary nucleic acids. Additionally, it has been utilised to identify drug metabolites in human saliva, such as methadone, 2-ethylidene-1,5-dimethyl3,3-diphenylpyrrolidine, antipsychotic medications, and antiepileptic medicines [16,17,22]. However, compared to biological metabolites, analytes recover less frequently [24,25]. This may be as a result of the high protein binding rates of some medications or the absence of denaturation in salivary protein. Therefore, we advise using the FTA Classic Card to determine the drug content in saliva. This filter paper increases the protein's ability to denaturate and increases the release of drug metabolites. The matrix effect should be taken into account in assays employing the DSS method because the chemicals on the filter papers alter the test findings when such filter sheets are used for saliva collection.

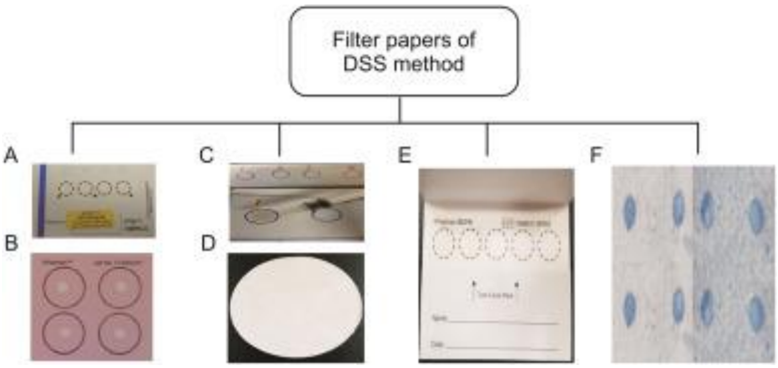

fig. 1 filter paper types used in the dried saliva spot (dss) method fig. 1: whatmantm 903 protein saver card (a), whatman ftatm dmpk-c card (b), color-indicating card (c), filter paper for determining lidocaine (d), filter paper for determining uric acid (e), and solid-material filter paper made of an alginate and chitosan foam (f) (reprint from refs. [18,23,26,33] with permission.)

Traditionally, biological fluids are poured over filter paper comprised of pure cellulose, just like for dry spot sampling. Blood and saliva samples have recently been collected and stored using soluble materials. For saliva sample, Lodoen et al. [33] developed a soluble substance made of an alginate and chitosan foam (Fig. 1F). Compared to filter sheets made of pure cellulose, soluble materials have two benefits. Both the sample preparation time and the analytes from the spot are transferred 100% of the time, respectively. Such filters are foams, which are naturally porous, swelling, and hydrophilic matrices that can quickly absorb biological fluids, making this conceivable. However, this unique soluble material type has several disadvantages. An additional step of analyte extraction or biopolymer precipitation is required before analysis since the extracted solution for analysis already contains the dissolved biopolymer and other foam components in addition to saliva components and analytes. DSS testing with foam-based soluble material therefore necessitates an additional dissolution step when compared to the use of standard fibre filter paper. But we think the preliminary results from tests using alginate and chitosan foams are really encouraging. Any difficulties involving exclusive diluents can be avoided by switching from conventional saliva collection to dry storage of saliva on sheets of solid materials. Investigation into soluble biopolymers as DSS sample media still need a lot of work.

According to the information above, filter papers utilised in the DSS method comprise plain filter paper, filter paper made from soluble components, and filter papers intended for spotting dried blood. This approach frequently makes use of the WhatmanTM 903 card, which can absorb saliva samples ranging from 25 to 100 mL. Due to the huge amount of saliva present, the sample cannot be completely dried in this situation. A hairdryer can be used to quickly dry the filter paper if only a small amount of saliva—say, 3 mL—has been dropped on it. This will shorten the drying period. Because drying takes between one and two hours on average, and even overnight for some samples, this is advantageous for everyday applications. The effect on the DSS and analytes' temperature stability should be taken into account simultaneously. As a result, in the future, the DSS method will necessitate the creation of filter paper made from more modern materials that are less expensive and require less saliva.

2.2. Types of filter paper cutting equipment

The fact that DSS is punched from filter paper is a well-known fact. The filter paper is cut using two separate types of machinery. The spots are typically removed manually using punching instruments as the Harris MicroPunch, Miltex® biopsy punch with plunger, and rapid-punch (Microscopy Products for Science and Industry) (Fig. 2A) [34]. These are constrained in that they can only punch one hole at a time and that the precision of the punching may decrease as more dried patches are examined. Alternatively, Johnson et al. [34] used a TOMTEC device (Fig. 2B) as an enhanced punching technique for semi-automated dried whole blood spots. When comparing the semi-automated methods' findings to those of the DMS methods that used manual punching and extraction, no statistically significant difference was found. Therefore, it was discovered that neither type of equipment (manual punching or semi-automated) had an impact on the results of the experiment. A manual punch is used in a recently released DSS paper; its idea form is depicted in Fig. 3 [26]. By requesting the user to open their mouth and place the collection tube on their lips so that saliva flows into the tube naturally, the entire amount of saliva can be collected. This procedure is easy to use and won't irritate the patient.

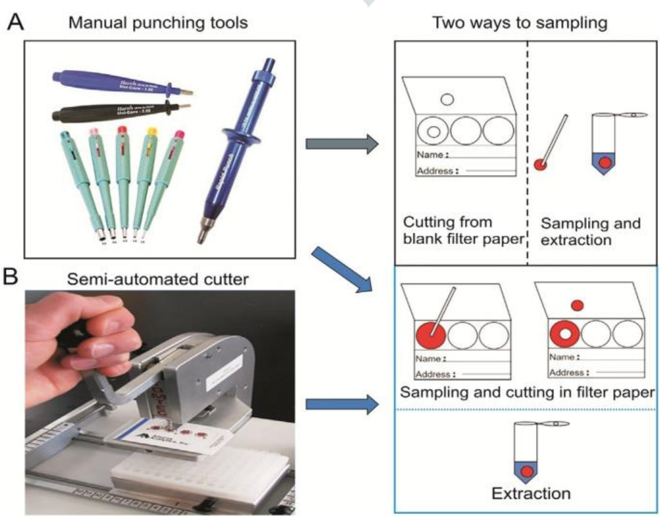

fig. 2. different types of equipment for cutting filter paper for dried saliva spot (dss) testing: (a) manual hole punch and (b) semi-automated cutter from tomtec. (reprint from ref. [34] with permission.)

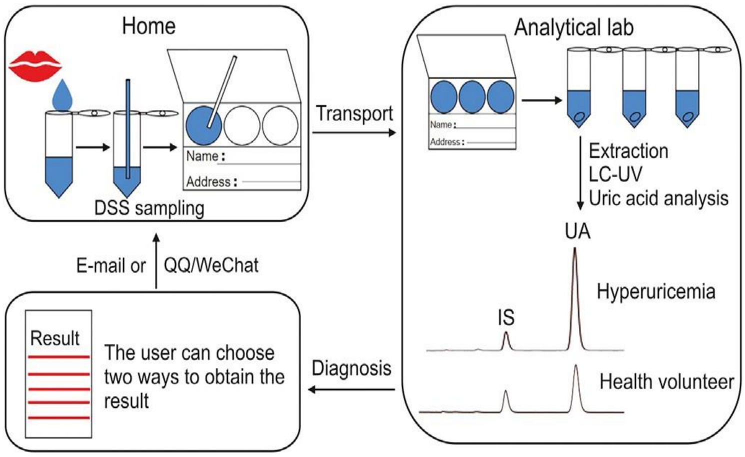

Fig. 3. Preparation and brief schematic of dried saliva spot (DSS) for determination content of uric acid (UA).

LC-UV: liquid chromatography-ultraviolet; IS: internal standard. (Reprint from Ref. [26] with permission.)

Inserting a collecting tube or other special device into the salivary gland is necessary for salivary gland collection. The latter operating procedure requires an expert to operate because it is difficult to operate and takes a long time to sample. Consequently, it is advised to use the first collection technique. Saliva can be moved from the collection tube to the filter paper using the capillary tube. The volume of saliva varies with the chosen filter paper, ranging from 3 to 100 mL, and different filter papers have varied diameters and liquid absorption rates. Following patient sample and drying, VAE extracts the dried saliva spot using a Li2CO3 solution. Utilizing the same solution, the extracted sample is concentrated and reconstituted. This particular procedure is used to find the presence of uric acid in human saliva. Our current thinking is that it is simpler to use the entire spot that contains the biological sample for analysis as opposed to poking a hole through it. It is preferable to cut the filter paper into areas before adding the saliva sample for two reasons: Dryed saliva and other clear biological fluids are difficult to find on filter paper without colour indication, and cutting the filter paper with the sample on it may cause an uneven distribution of the analyte concentration in dried regions. There is no doubt that both of these techniques will add to analysts' workloads. Therefore, the filter paper can be divided into spots of specified dimension and inserted one spot per well in a 96-well plate. This spot is sent to the analysis lab via mail after the user places the sample on it and lets it dry. Cutting tools are no longer required of analysts, which not only lessens workload but also guarantees the accuracy of analyte concentration.

2.3 DSS sample extraction techniques

Evaluation of extraction techniques should follow the creation of adequate filter paper and the choice of the proper cutting instruments. Using a rigorous research, the viability of the extraction procedure was confirmed. The stability of the analyte on filter paper should also be taken into account for practical applications. We therefore list the main tactics in this part, such as the inclusion of an internal standard (IS), enhancement of the extraction method, and assurance of analyte stability. 2.3.1. Including an IS in the DSS analysis

2.3.1. Including an IS in the DSS analysis

Table 1 lists articles that used the DSS procedures [11–26] and added an IS to the extraction solvent as opposed to dumping it directly onto the filter paper. In contrast to Zimmer et al[35] .'s recommendation that IS be added to the filter paper prior to the biological sample during the preparation of the DMS sample, Zheng et al[23] .'s recommendation that IS be added to the extraction solution and for the DSS sample to be extracted after the analyte was eluted (Fig. 4). Making sure that the IS and analyte have equivalent extraction efficiency is crucial. Manicke et al. [36] and Christianson et al[27]. The average precision (coefficient of variation,%) and accuracy (%) of adding IS before or after putting the biological sample to the filter paper were compared. In both investigations, it was discovered that adding IS to the filter sheets before to the biological sample increased the method's precision and accuracy. Since IS corrects for analytes from the collection paper during the punching process, we think that this gain in accuracy and precision is due to this one factor. Therefore, for DSS, we recommend using the complete, indicated circular region of the sample rather than punching discs from the filter paper containing the saliva sample. This has two benefits: 1) if the full collecting site is used, one need not worry about the saliva's uneven distribution on the filter paper when punching holes in the sample card; and 2) IS does not account for the analyte because the complete saliva sample is used during extraction for DSS. Additionally, since the diameter of the filter paper spot can be altered in accordance with the volume of the sample, cutting out the filter paper before adding the saliva sample is also a good strategy. This method uses less filter paper and speeds up the drying process for the sample.

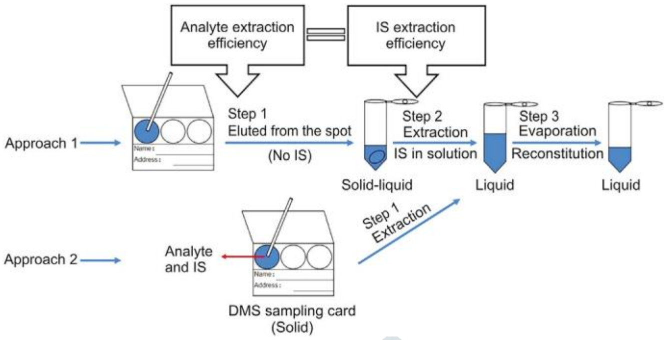

fig. 4 shows the extraction steps for a dried matrix spot (dms) sample. internal standard (is) (reprinted with permission from references [23,35])

2.3.2. Analyte stability in DSS

Reliable quantification depends on the stability of the sample throughout the whole analytical process [39]. There are four categories of analyte stability: autosampler, freeze/thaw cycle, short-term, and long-term (Table 1). It is crucial to understand that instability can occur in both the sample matrix and the processed samples. As a result, it's crucial to check the stability of analytes in samples made with an autosampler. In order to establish how long dried saliva can be regularly stored and under what circumstances it must be sent to the laboratory for analysis, it is crucial to understand the short-term and long-term analyte stabilities.

Some researchers used filter paper as samples in the DSS approach by applying thawed frozen saliva to it [16,17,22]. Since saliva contains salivary amylase, freezing will either cause the analyte concentration to change or cause it to precipitate. Therefore, it is necessary to analyse the analytes' freeze/thaw stability across a minimum of three freeze/thaw cycles at three concentrations in triplicate. However, because it requires the patient to complete an additional step at the time of sample collection, this technique of freezing saliva at the time of collection is not suitable for the on-site collection process for DSS. This makes it problematic for widespread adoption and regular use.

2.3.3 Instruments with high sensitivity for analysis

In 2014 and 2015, respectively, the first uses of the DSS sampling approach for TDM and illness diagnosis were reported by Abdel-Rehim & Abdel-Rehim [18] and Numako et al. [12]. The relationship between detection sensitivity and sample volume is still difficult to understand even after years of improvement. High performance liquid chromatography (HPLC) connected to either a photodiode array (PDA) or UV detector was used in several experiments to detect analytes (Table 1). For the DSS analysis employing the PDA detector, the limit of detection (LOD) was 0.03 mg/mL. However, employing tandem mass spectrometry (MS/MS) detection, the LOD was 6.75 fg/mL level. In addition, we discovered (in this review) that the minimal volume for a biological sample to be detected by MS/MS was 3 mL. The demand for bio-sample quantities in DSS methods has decreased due to the enhanced sensitivity and selectivity of these detectors. MS/MS detection provides better sensitivity and permits a wider linear detection range as compared to UV detection [40]. It has significant advantages in both qualitative and quantitative aspects, and it can also provide relative molecular mass and structural information [41]. However, MS detectors are more expensive than UV detectors, both in terms of acquisition and maintenance, and the operation of an MS requires a qualified analyst with specialised training and knowledge.

3. Additional dry spot technique tactics

Along with the DSS method, filter paper is also used in the DBS and DMS processes for shipping dried sample spots, including blood, plasma, serum, urine, and herbal remedies. Widespread reporting of these samples found on filter papers has encouraged the development of such techniques. Due to their ease of use, convenience, robustness, and dependability, such sampling techniques have drawn a lot of attention.

3.1 DBS and dry plasma spot (DPS)

Phenylketonuria illness was identified using the DBS approach in human newborn screening in 1963 [42]. Since then, the use of this technique for streamlining sample collection and processing has developed quickly in the context of illness diagnosis and newborn screening. Currently, the use of DBS has spread to a variety of applications, including extensive epidemiological research [43], testing for hepatitis C (HCV) [48] and coronavirus illness 2019 [47], as well as neonatal genetic screening [49]. DBS has a number of benefits over traditional sampling methods, including the following: 1) a lot less blood is needed; 2) collection and preparation costs are low; 3) the method is easy to use and reliable to perform using peripheral blood from fingertip or heel pricks with little trauma and little discomfort, which is readily acceptable to the patient; 4) remote sampling is allowed and can even be done at home; 5) low transportation and sample storage costs; and 6) there is no pollution risk to the environment [50,51]. The expense of creating new techniques based on DBS technology is considerable, however, and a patient's hematocrit can have an impact on a DBS sample. This has an impact on the diffusion area of the DBS and has a significant impact on the con- centration assessment of target chemicals [52]. Additionally, there is a chance of low sampling quality. It can be difficult to offer patients consistent, uniform, high-quality areas for at-home sample. Additionally, DBS technology encounters false-negative and false-positive outcomes for anti-HCV screening.

As was already indicated, DBS has various limitations, chief among which is the bias in the results brought on by the presence of erythrocytes in whole blood [53]. Plasma is a better sample for examination because it doesn't contain red blood cells [54e60]. In TDM [61e63], illness diagnosis [64e66], monitoring antiretroviral therapy [67], and clinical pharmacokinetic research [68], this DPS sampling approach is now employed extensively. The advantages of the dried spot method are offset by the need for specific equipment and personnel for plasma collection. Microwave-assisted extraction and a dried plasma spot were coupled by Brahmadhi et al. [69], significantly speeding up the processing of the material. However, to obtain plasma using this process, expert machinery is needed. Whole blood was collected using the cobas® Plasma Separation Card by Marins et al. [70], who then compared it to plasma samples. This study addressed the issue of using specialised tools for plasma preparation. However, compared to the sample amount needed when utilising the DBS approach, whole blood samples are bigger, which raises the risk of infection for patients. Gao et al. and Ryona et al. [71] and [72] created a non-solvent-thermally induced phase separation (N-TIPS) and a book-type dried plasma spot card, which require a smaller blood sample and can be used in patients who are unable to give samples themselves.

3.2. DMS

In DMS sampling, a dye is added to the collection paper to mark the locations of the dried sample spots, a DBS application that is employed in non-blood matrices [73,74]. It is challenging to locate the visual spot and to ensure precise removal and extraction of the sample spot for testing when a transparent or almost transparent analyte, such as cerebrospinal fluid, saliva, tears, or synovial fluid, has dried on the collecting paper. Therefore, to aid scientists in visually verifying the location of the dried sample, a color-indicating dye is put to sample collection cards. This technique guarantees a trustworthy and dependable collection of transparent fluid samples, enhancing the testing's accuracy and precision. The use of a colour indicator in DMS, as opposed to DBS, to identify the location of dried sample spots complicates the technique and adds time. Additionally, not every analyte may be compatible with a color-indicating dye because the target analyte's selectivity may be impacted by the high dye concentration in the area. Therefore, it may be difficult to select a dye that is appropriate for DMS sampling.

The most common physiological fluids in which metabolites can be identified include blood, plasma, serum, and saliva; however, urine is also a popular sample that is analysed. Blood is the most common physiological fluid in which metabolites can be found. In contrast to the flow of blood and lymph, the circulatory system is made up of a number of different components.

The collection of plasma and urine is a straightforward and painless process.

Urine is being utilised to detect toxins, evaluate treatment compliance, and identify illegal compounds [75,76]. Urine is also being used to identify illegal substances. A DUS, or urine sample collection device, is a container that holds filter paper and collects urine samples. After that, the substance is diluted to an appropriate degree before being put on filter paper. When using filter paper, it is important to ensure that the volume of the sample that is deposited on the paper does not exceed the maximum loading capacity of the filter paper. After the spots have been obtained, they are allowed to dry at room temperature or the temperature required for a considerable amount of time or overnight. After punching a complete DUS off the card, the analytes are extracted with a predetermined volume of organic solvent using VAE and UAE. This process is repeated several times. Once the solution has been dried, it will be redissolved in the mobile phase. Determination needs to be made concerning the extraction solvent, the volume of the solvent, the amount of time spent extracting, and the amount of time spent drying. Filter papers such as the Whatman® 903 protein saver card [77,78] and the Whatman FTA classic card [60,79] are frequently used for DUS and are analogous to those used in the DSS technique.

In recent years, DUS has been put to use in the diagnosis of a wide variety of conditions, including type 1 glutaric aciduria [80], congenital CMV infections in infants [81], and human HPV testing [82]. Because of this, the focus of future development of DUS methods will continue to be on filling in the gaps between the many existing DUS instances found in TDM applications.

The analysis of dried spots of herbal medicines, also known as DSHM, is carried out using the same method as that used for dried spots of biological materials [83]. The method is laid out in flowchart form in figure 5, which can be seen here. A filter holder has specks of a dried substance placed in it so that it can be processed in real time. This apparatus is positioned in front of the chromatographic column, and it enables online sample extraction by allowing the mobile phase to flow through the dried spot. In contrast to the offline elution that is performed in a traditional DMS sampler, the analyte is eluted online in the DSHM method using an in-line filter holder. Despite the fact that at first glance this method may appear to be more effective than others, it cannot be used for the analysis of enormous amounts of data.

Recent advancements in paper-spray mass spectrometry, when combined with DSS and DUS, make it possible to directly ionise analyte from a biofluid spot on the paper. Take into account the following: Bills et al. [24] presented a concentration and maintenance strategy.

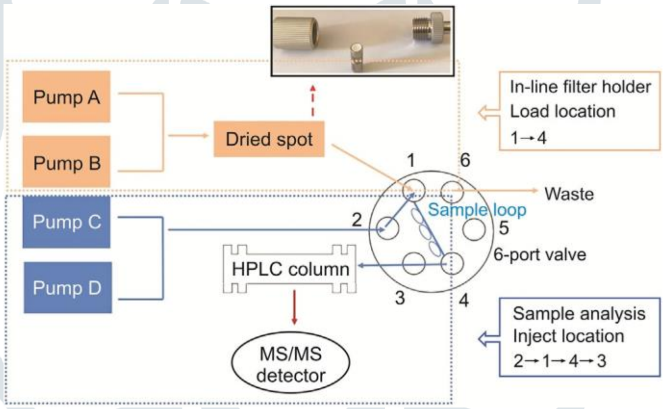

fig 5: schematic illustrations of the dried herbal medicines spot (dhms) method. high performance liquid chromatography is abbreviated as hplc. (with the author's permission, reprinted from ref. [83])

collecting urine and saliva samples that contain tetrahydrocannabinol (THC) and synthetic cannabinoids for analysis by paper-spray mass spectrometry using paper strips. In order to extract the sample using a paper strip, the sample is run through an oil spot that is printed on a 40 mm paper strip. In order to verify the consistency of the paper, it is frequently cut into strips that are only 5 millimetres broad. THC is famously difficult to detect in bodily fluids like urine and saliva due to their low concentrations.

Because of this, the analytes were preconcentrated in sesame seed oil so that the analysis could be completed more quickly. The overall sensitivity of the two methods for detecting THC in urine and saliva was brought down to less than 20 ng/mL as a result of the reduction in sensitivity. This method would be particularly useful for the detection of substances in unstable matrices in the future, such as saliva and other biosamples, and would be of great benefit to the process. When these direct analytic techniques are utilised, it is possible to considerably boost the throughput of DSS bioanalysis. Paper spray has been shown to have good sensitivity and a quick analysis time, which makes it a practical option for TDM as well as disease detection.

Isomer discrimination utilising approaches based on ambient ionisation continues to be challenging despite the fact that skipping the extraction phase has a number of advantageous side effects.

4. Conclusions and future perspectives

We explore the current deployment of the DSSmethod and its shortcomings in sample preparation and validation using examples from both TDM and disease diagnostics. Specifically, we focus on the flaws in the DSSmethod's sample preparation. Researchers that use this technology are given access to a thorough analytical technique as well as ideas for additional research. An entirely new field of research and development has been begun as a direct result of the availability of this uncomplicated, efficient, and trustworthy method of sampling. In addition, the techniques of DBS, DPS, DUS, and DSHM have been applied in order to identify samples of blood, plasma, urine, and herbal medicines. In the fields of biology and botany, the techniques for dry spot analysis that were mentioned earlier can be applied in a variety of contexts.

At the moment, the vast majority of commercial filter sheets that are utilised in the collection of alternative biological fluids are insufficient. It is unavoidable that new kinds of filter paper will be invented as these methods grow more prevalent across the world. Because the direct analysis approach does not require the time-consuming step of using filter paper for extraction, it is ideal for applications in which efficiency is of the utmost significance, such as those that take place in everyday life. In addition to this, it sets the way for the future development of straightforward and quick methods for dry spots. In most cases, the volume of the sample that is being determined is one microliter in order to make the process less invasive and to make drying easier. Therefore, it is difficult to achieve a high degree of sensitivity with either the DSS method or the dried spot of various biological fluids. Both of these methods have their advantages and disadvantages. The vast majority of the sample examinations were carried out in the confines of a laboratory. In situations that take place in the real world, the collection of samples by patients or end-users is likely to provide issues due to difficulties with efficiency and precision. People may soon be able to learn about their health without the inconvenience of having to leave the comfort of their own homes as a direct result of the rapid growth of these approaches.

CRediT author statement :

Yu Han was responsible for the following: the conceptualization of CRediT; the formal analysis performed by Yu Han; the investigation conducted by Minghui Zhang; the analysis performed by Minghui Zhang; the analysis performed by Jing Wang; supervision provided by Su Zeng; visualisation performed by Su Zeng; data collection performed by Jun Zhe Min; drafting performed by Jun Zhe Min; review and editing performed by Yu Han; Yu Han's acquisition of financial resources.

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There is not a single conceivable source of bias, as far as the authors are aware.

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