The availability of more sensitive mass spectrometry (MS) systems has extended the use of dried blood spot sampling to biopharmaceutical applications. For about 50 years, dried blood spots (DBS) have been used in routine neonatal diagnostics for the determination of metabolic disorders – such as phenylketonuria – with bacterial growth inhibition assays (1). With the DBS sampling technique, small volumes of sampled blood, usually 10–20 μl, are spotted onto filter paper sampling cards. These cards, or usually sections of the cards containing the samples, are then used for downstream analysis.

In recent years, the technology has seen increasing interest in a number of applications – such as forensic reference DNA data basing and molecular diagnostics – mainly using downstream PCR and PCR product analysis. One of the most widely discussed applications is the use of DBS for drug metabolism and pharmacokinetics (DMPK) studies in the pharmaceutical industry for preclinical and clinical research. The most important development to facilitate the use of DBS in drug research has been the recent availability of more sensitive mass spectrometers. This new generation of mass spectrometers has enabled attainment of the lower limits of quantification that are crucial for the more limited volume of analyte contained in DBS samples. Currently, many pharmaceutical companies and contract research organisations (CROs) have started
to actively investigate DBS for use in preclinical and clinical trials, and several studies have shown the robustness of DBS analysis for different compounds (see for example (2)).

The same technology as for DBS can be used for dried matrix spots (DMS); these are liquid matrices – for example, body fluids such as plasma but also rare body fluids such as tears or spinal fluid – that are dried on sampling paper. This means that a completely new set of data is available for clinical studies.

**Benefits of DBS Sampling**

Compared with plasma sampling and analysis, DBS sampling on cards significantly reduces the volume of blood required for analysis. This reduction allows the realisation of two main ethical benefits: first, a reduction in the use of animals in preclinical studies; and second, more ethical sampling from children or seriously ill patients.

For preclinical studies, animals are used for the testing of pharmacokinetics and toxicology. With DBS, the lower volume of blood sampled at each time point means that serial, rather than composite, toxicokinetic (TK) profiles can be obtained from individual animals, as the potential deleterious effects of sampling higher volumes of blood are eliminated. So DBS sampling results in a significant reduction in the number of animals required, and therefore offers ethical advantages in terms of the reduction and refinement of animal use.

For clinical studies, DBS testing is less invasive as it usually requires only peripheral blood to be sampled. Together with the reduction in volume, this enables the ethical sampling of patients such as children or the seriously sick, for whom sampling larger volumes might pose a health risk.

Besides the ethical benefits, economic benefits are an important driver of the wider use of DBS technology. While the processing costs are comparable with liquid plasma samples, shipping costs are significantly reduced because packing of the sample in dry ice is no longer necessary. This could lead to potential savings of several 100,000 euros per clinical study – making the technology very attractive for pharmaceutical companies (3).

**Current Technologies for DBS Analysis**

Several technologies are currently available for DBS analysis, each one offering its unique benefits and drawbacks. The standard and most common method resembles the Guthrie method (1). The sampled cards are punched and the resulting paper sections are collected in well plates for subsequent extraction – either manually or in a liquid handling system. The sample is then analysed in a mass spectrometer.

The punching method offers the highest flexibility as it allows for different punch sizes and extraction conditions – for example, different extraction solvents or temperatures. In addition, sample dilutions such as ‘doughnut punching’ can be performed; this uses smaller punches of sample joined by punches from sample without compound, so all samples to be extracted will have the same volume of matrix.

Direct elution or online extraction represents a more direct method. For this, the card is clamped into an adapter of a defined size that works as a front end for a liquid chromatography-mass spectrometry (LC-MS) system. The card is integrated in the LC flow path for extraction and purification before being inserted into the mass spectrometer. The technology basically resembles thin layer chromatography (TLC)-MS systems – just on cards. Direct elution removes the requirement for separate extraction of the sample, and enables direct connection to a mass spectrometer. However, the technology faces certain challenges as its flexibility is limited. In addition, the complexity of DBS samples challenges the capacity of the columns used.

Other technologies are currently being tested – one being desorption electrospray ionisation (DESI), where the sample is directly ionised from the source card into the mass spectrometer. DESI removes the requirement for additional steps and is therefore the most direct method, but throughput is currently very limited.

**Challenges of Automating DBS Analysis**

The transfer of manual processes to an automation mode always brings a number of challenges. Automation is required to increase the throughput of samples in analysis to make the method suitable for certain processes. However, automated systems lack the flexibility of a human operator to respond to inputs that might be out of specification. Thus they require strict monitoring of input samples and quality control of processes.
For dried blood spots, an increase in throughput is crucial for the method to be widely adopted for preclinical and clinical studies. On the other hand, the quality of the card and sample vary widely depending on the treatment. This is especially true when samples are obtained in a variety of places, challenging the robustness of card handling and analysis. So all high throughput applications currently in development would face similar challenges, namely the robustness of card handling (including avoidance of cross contamination and carry-over), and analysis of the card and sample to enable reproducibility in processing and documentation.

Robust handling of sample cards is crucial for the automation of dried blood spots. Two card types are currently available from several suppliers. The first consists of just a printed piece of filter paper that, due to its very limited robustness, gets deformed quite easily during sampling, shipment or storage. This can lead to problems in handling the cards reliably. The second card incorporates a cardboard frame that increases the robustness of the system and can thus be automated. Nevertheless, inappropriate treatment during sampling, shipment or storage can still lead to bending or deformation, impairing the reliability of the system. In a worst-case scenario, the card might fail to be delivered to its respective position, crashing the system and causing hardware errors. For cases such as this, an error handling strategy has to be implemented; for example, the Hamilton easyPunch system incorporates an error-handling system whereby deformed cards are recovered into special magazines.

More robust frames that cannot bend – such as those made from plastic – could avoid these problems, as they would be strong enough to withstand poor treatment; however, such frames are not widely available for the DMPK market.

A potential second problem is carry-over between cards. For shipment and storage, all cards are usually placed in separate single-use pouches, while for the automation of card handling, different kinds of magazines are used to keep the cards in predefined positions. These magazines require a guide to retain the cards in position; this just needs to cover the frame of the cards, but should avoid crossing the sampling area. If cards are bent, then parts of the magazine might cross the sampling area, causing sample material to be transferred to subsequent experiments, potentially impairing the results. A possible solution is to use magazines that only have a guide around the cardboard framed sides of the card. This prevents any touching of the sampling area by other cards or parts of the magazine, and therefore carry-over between different experiments is avoided.

Another potential source of carry-over relates to the device that extracts the defined size of the sample – that is, the punch piston. In this case, carry-over can be caused by sample material sticking to the piston. Two methods of cleaning strikes can be employed – either on the same card as the sample, or on an empty card, in order to avoid carry-over.

Sample Analysis

Reliable analysis of the sample card is key to guaranteeing reproducible results. Although commercially-available cards do have special sampling areas, the sample spot might not be centred in these areas, or the punch or extraction size might not cover the whole area of the spot. DMS on indicating paper presents an additional challenge. Most of the time, samples can barely be seen, as most of the additional body fluids used for DMS are colourless. Only when they are sampled on indicating paper does the colour change when probed. So, imaging capability is required not only to trace a
Another important aspect is the traceability of samples. Here, two processes are required: first, tracing the barcode of the card and the sample itself in downstream operations; and second, documentation of the sample in the process.

One potential solution to this is to use a system that incorporates a charge coupled device (CCD) camera to identify the cards and the samples on them. It then determines the punch positions according to pre-set parameters. Different punching positions can be chosen relative to the sample. The CCD camera also enables the recognition of colour change due to sampling on indicating cards.

Avoidance of Bias

While separate discussions are going on about sampling of DBS and the best way to do this, automation solutions have to deal with samples that might be invalid due to sampling errors, such as double sampling on the same field or blood smear. Automated detection of these errors and automatic rejection of invalid spots will reduce the variability of spots used – leading to lower variation and higher robustness, independent of any operator.

Sampled cards are defined by over 100 parameters that can be modified to adapt to the sample of choice. The settings can be modified to discriminate impartially between valid and invalid samples. In contrast to manual handling, there is no bias from the operator – enabling a better comparison between different experiments and sites.

Another important aspect is the traceability of samples. Here, two processes are required: first, tracing the barcode of the card and the sample itself in downstream operations; and second, documentation of the sample in the process. With the Hamilton easyPunch, the sample analysis step is used to read the barcode and trace the sample, and then after the punch, the disc is traced in the receiving plate using the camera function. Downstream processing, such as pipetting extraction reagent, will also be monitored. Both results are stored in a report file and the pictures are stored in a database; both sources can also be exported to a LIMS system for documentation.

Conclusion

Adaptation of DBS to pharmaceutical bioanalysis has significant ethical and economic benefits compared with classical liquid plasma samples. Several systems are available that enable DBS processing in line with the requirements of biopharmaceutical analysis. Current developments in the technology are focused on the robustness of card handling, the avoidance of carry-over, the robust and unbiased analysis of samples, and the traceability of the whole workflow. Solutions to these problems are required in order to have a system suitable for high throughput analysis. Use a system that addresses the challenges of DBS automation, including: a card handling system that deals with deformed cards and avoids carry-over; flexible and robust card analysis that avoids bias; and samples and actions that are monitored and traced in a report file that can be exported to a LIMS system.

References

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