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Dried Blood Spots in drug development

Background

Dried blood spots (DBS) refers to a blood sampling technique where small volumes of blood are spotted on an appropriate filter paper, dried, and taken to the labratory for analysis. The technique is well established in clinical labs for applications such as neonatal screening for inborn diseases, but has recently experienced a surge of interest in the context of drug development, i.e. toxicokinetic and pharmacokinetic studies.

DBS – Milestones

It would not be fair to call DBS as such a recent innovation. It is Guthrie et al. who introduced DBS as long back as 1963 in the context of neonatal screening of phenylketonurea. This approach has had a massive success for many years, and heel pricks of neonates with sampling on DBS cards are practised up to today in many countries worldwide. A significant body of experience with DBS is available from that angle. The U.S. Food and Drug Administration (FDA) has already registered two sources of filter paper for blood collection as Class II Medical Devices (21 CFR §862.1675) based on sustained compliance with the performance parameters specified in the Clinical and Laboratory Standards Institute (CLSI) LA4-A5 Approved Standard and these have been tested extensively in the context of neonatal screening (CDC, 2009).

DBS sampling approaches for drug development are amenable to the same types of bioanalytical detection principles as traditional plasma or whole blood samples, i.e. HPLC-UV (Soons et al., 2006), LC/MSMS (Barfield et al., 2008), GC/MS (Déglon et al, 2010). Microbiological assays have been used based on DBS sampled material for folate screening (O'Broin & Gunter, 1999). Thyroid hormones are an example of biomarker that have been analysed by immunoanalytical methods for many years in neonatal screening, which indicates clearly that the technique is amenable to ligand binding approaches (CDC, 2009). Various biomarkers and even immunogenicity assays have been performed with the DBS format, but here it is worthwhile to note that particular attention should be given to the selection of the filter paper in the development of such assays. Most historic DBS work was based on filter papers that contained denaturing agents, inactivating bacteria and viruses, which contributes to safety and stability. However, these types of filter paper denature proteins, and as a consequence may destroy epitopes necessary for binding in a ligand binding assay, or render them inaccessible. It is therefore recommended to use non-denaturing filter paper and solvents that are compatible with protein work when attempting to quantitate proteinaceous compounds (biopharmaceuticals, biomarkers, anti-drug antibodies) in DBS.

Benefits of using the DBS sampling technique

The process of plasma preparation from whole blood is the separation of the cellular fraction of the blood from the plasma fraction by centrifugation. This process implies discarding approximately half of the original whole blood volume. It is clear that bioanalysis based on whole blood makes in principle more efficient use of the sample obtained from the test animal, volunteer or patient. This is more than relevant in a number of cases, as follows.

Toxicokinetic study designs in rodents are often based on a main group of animals that is assessed for toxicological effects of the adminstered drug, and a satellite group of animals that is used for pharmacokinetic assessment. The low volume DBS strategy can mean that in many cases the blood sampling is done directly from the main group of animals, without impact on their well-being and on any potential toxicological observations. Without the need for satellite group animals, this approach reduces the number of test animals in TK studies, which is an obvious ethical and cost-related advantage.

Furthermore, as the DBS approach consumes less blood per timepoint than a traditional blood to plasma strategy, it is possible to collect pharmacokinetic profiles from single animals, rather than having to draw conclusions from composite profiles from different animals. The consequence is that better quality TK data is obtained.

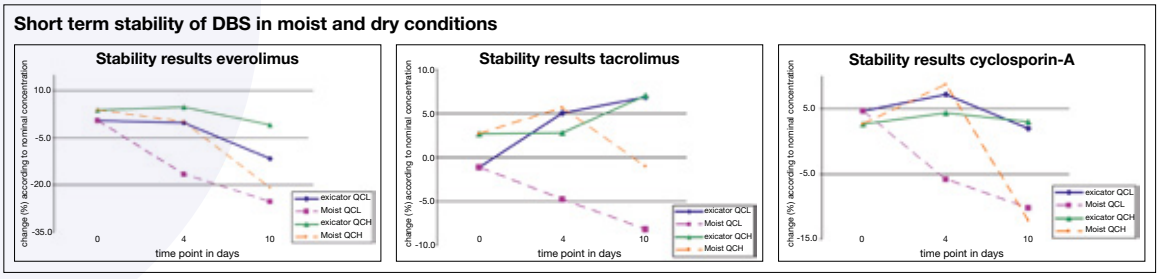
The low blood volume requirements don't play as much for large animal species and for humans, with the exception of critically ill, special patient and pediatric populations. The advantage for pediatric applications is excactly the same as the one that was seen 40 odd years ago when heel pricks were introduced; it is both ethically and medically not justifiable to draw large volumes of blood from neonates or infants.

In the clinic, a sampling strategy based on DBS means that no centrifuges are required for preparing plasma, and no freezers for storage prior to shipping to the bioanalytical facility.

As a central lab organisation, we at Eurofins see large volumes of dry ice being shipped around by courriers for the transport of frozen biological samples. Especially in the case of late phase clinical studies, multiple clinical sites are often involved, sometimes located in various continents. These are bulky transports that require special care, and are therefore costly. In terms of logistics, DBS offers a huge simplification. The dried filter papers can be shipped at ambient conditions in a simple envelope. We have demonstrated in our lab that

humidity in particular is a factor that can interfere with the reliability of the bioanalytical assay. We therefore recommend that filter papers be shipped protected from environmental humidty, i.e. in a plastic pouch and preferably with some desiccant material. The modest material requirements for the clinic (no centrifuges, no freezers), and the simplicity of the logistics are obviously attractive to centers all over the world, but particularly in areas where these resources are less easily available. DBS sampling has for example been succesfully used for HIV screening and clinical development strategies in Africa.

Whereas initially many concerns were raised concerning the stability of the analytes of interest in a DBS setting, these concerns were in most cases unwarranted. On the contrary, it has been seen repeatedly that compound stability is at least as good in DBS than in plasma or frozen whole blood. Furthermore, glucuronide metabolites were also found to be unexpectedly stable.



Disadvantages?

The one area where DBS complicates things is in the bioanalytical lab. Where the preparation of plasma from whole blood can in itself be considered a sample preparation with the removal of the cellular particulate matter, receiving a dried blood spot as starting material is a different ballgame alltogether. Extracting the analytes of interest that are trapped in denatured proteins present in a dried spot on a filter paper that itself presents previously unknown interferences requires careful optimisation. While some of the basic questions such as impact of blood volume spotted, impact of spotting with capillary or pipette, etc have been addressed (Spooner et al., 2009), aspects like the optimisation of the extraction, the selection of the type of card type are additional DBS-specific questions that need to be looked at on a case by case basis.

DBS in drug development

While not a disadvantage per se, we have to bear in mind that the underlying matrix in DBS is not plasma, but whole blood. Traditionally, the preferred matrix for PK and TK studies has been plasma, which is a matrix relatively easy to prepare, and to some extent

more amenable to the majority of analytical techniques than whole blood. As a consequence, pharmacokineticists and regulators are more familiar with dealing with plasma data than with whole blood data. It may not make much sense to introduce DBS for compounds that have a large body of established knowledge that is based on plasma data.
The balance has to be made on a compound to compound basis, but starting as early as possible in the drug development route with DBS certainly sounds the more sensible option. When switching from plasma data to DBS data during the development course may require additional work to bridge the two types of data.

DBS applications

Based on the advantages mentioned above, it is likely that DBS will find its place in the preclinical drug development. There are published accounts of such studies, providing proof of concept, and it is clear from presentations at dedicated DBS sessions during meetings by DIA, AAPS, EBF and other organisations during 2009 and 2010 that a significant number of innovator pharma companies are evaluating the technique in their own drug development programs. GSK has been spearheading the recent interest in DBS for drug development applications. As early adopters, they use DBS as the recommended analytical approach for the assessment of PK/TK data for all new oral small molecule drug candidates, which have previously demonstrated a successful bioanalytical validation (Barfield et al., 2008).

As the benefits of DBS are not restrained to preclinical studies, a DBS strategy can be considered for clinical phaseI and IIa studies as well. As in the case of TK studies, nurses and other study personnel will have to be trained, but feedback from the staff is generally favourable to DBS based sampling.

Another interesting area is therapeutic drug monitoring (TDM), where circulating drug concentrations need to be monitored, typically for drugs with a narrow therapeutic window and/or large inter-subject variability. This area often involves clinical laboratories, and it is interesting to see that DBS has been used longer in TDM than in TK and PK studies. Examples include the monitoring of metformin in diabetic patients, which reportedly has been done with DBS (AbuRuz et al., 2006) or the immunosuppressant everolimus in transplant patients (Van der Heijden et al., 2009). Eurofins Global Central Laboratory advanced further on the path of immunosuppressants and offers therapeutic drug monitoring based on DBS for combined LC/MSMS analysis of everolimus, tacrolimus and cyclosporin A (unpublished).

Future developments

While manually punching and extracting is acceptable for small sample numbers, this is a laborious step that calls for automation. Various technological options are available or under development. Semi-automated punch robots are available that drop the punched material in a 96-well plate format. From there, extraction can be performed.

An approach that cuts out the punching step alltogether is directly interfacing the DBS filter paper to the LC/MSMS, i.e. extracting on-line. Various attempts are made in this direction, including the use of a TLC extraction device (Camag) that elutes the spot, with the outlet interfaced directly to the MS. Alternative approaches start to be published (Kertesz & Van Berkel, 2010), so it is more than likely that instrument vendors will come on the market with proprietory interfacing solutions. It is likely that these will become available in the not too distant future.

Conclusion

DBS as a blood sampling technique for applications in drug development is a recent phenomenon, spurred by financial and ethical motivations. It is remarkable that the power of modern analytical technology has allowed an old sampling technique to become prominent in a short period of time. In view of its advantages compared to traditional plasma-based strategies, it can be expected that DBS is here to stay, certainly if DBS-based NDAs or submissions pass regulatory scrutiny.

References

AbuRuz, S.; Millership, J.; McElnay, J. (2006). Dried blood spot liquid chromatography assay for therapeutic drug monitoring of metformin. J. Chrom. B, 832:202–207.

Barfield, M.; Spooner, N.; Lad, R.; Parry, S. & Fowles, S. (2008). Application of dried blood spots combined with HPLC-MS/MS for the quantification of acetaminophen in toxicokinetic studies. J. Chrom. B. 870:32-37.

CDC (Center of Disease Control) (2009) Newborn quality assurance program – filter comparison study. http://www.cdc.gov/labstandards/pdf/nsqap/nsqap_FilterPaperStudy51809.pdf

Déglon, J. ; Lauer, E.; Thomas, A.; Mangin, P. & Staub, C. (2010). Use of the dried blood spot sampling process coupled with fast gas chromatography and negative-ion chemical ionization tandem mass spectrometry: application to fluoxetine, norfluoxetine, reboxetine, and paroxetine analysis. Anal Bioanal Chem (online publication).

Kertesz, V. & Van Berkel, G. J. (2010). Fully Automated Liquid Extraction-Based Surface Sampling and Ionization Using a Chip-Based Robotic Nanoelectrospray Platform. J. Mass Spectrom.,45:252-260.

O'Broin, S. & Gunter, E. (1999). Screening of folate status with use of dried blood spots on filter paper. Am. J. Clinical Nutrition 70:359–367.

Soons, J.W.P.H.; van Bree, M.L.; Coumou, J.H. & Hulsman, J.A.R.J. (2006). Lamotrigine in dried blood spots by HPLC. Ned. Tijdschr. Klin. Chem. Labgeneesk. 31:238-239.

Spooner, N.; Lad, R. & Barfield, M. (2009). Dried Blood Spots as a Sample Collection Technique for the Determination of Pharmacokinetics in Clinical Studies: Considerations for the Validation of a Quantitative Bioanalytical Method. Anal. Chem.81:1557–1563.

van der Heijden, J.; de Beera, Y.; Hoogtandersa, K.; Christiaans, M.; de Jong, G.J.; Neef, C. & Stolk, L. (2009). Therapeutic drug monitoring of everolimus using the dried blood spot method in combination with liquid chromatography–mass spectrometry. J. Pharm. Biomed. Anal. 50:664–670.

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