

Minimising Volume, Maximising Returns

Sally Hannam, John Allinson and Ray Briggs at ICON Development Solutions discuss the approaches and advantages of reducing sample volume in clinical studies

Reducing blood sample volumes for biological analysis in drug development has been a driving force within the pharmaceutical industry for some time. The desire to include a larger range of analytical investigations, particularly biomarkers, into clinical studies has increased the pressure to use the limited sample volumes available for analysis more efficiently. Recently, there has been increased interest in the use of dried blood spot sampling (DBS) as a method of reducing sample volumes in toxicokinetic and selected clinical studies. Here we will describe the origins of DBS, its advantages and limitations in clinical research, as well as the most promising potential applications of other technologies for sample conservation that are available to the bioanalyst.

DBS: PRACTICAL ADVANTAGES

Dried blood spot sampling is a technique that has a long history. Guthrie *et al* introduced it to widespread medical practice as a screening tool for phenylketonuria in the early 1960s, leading to other applications in clinical pathology (1). While the technique did not achieve widespread adoption, it has more recently been championed by Spooner *et al* for the determination of drugs and metabolites in biological samples using manually extracted DBS coupled to LC-MS/MS (2). This approach to sampling is now being evaluated by a number of leading pharmaceutical companies for its promise in helping address the high sample volume, labour intensive and logistical challenges typically associated with this type of analysis.

DBS offers very significant practical advantages over traditional sampling methods. The samples are easy to obtain with a simple finger, ear lobe or heel prick, and blood can then be spotted onto a suitable commercial sampling paper. These papers act in rather the same way as blotting papers, absorbing the blood sample and distributing evenly through the paper to leave a spot of blood which is allowed to dry *in situ*. Samples can therefore be taken very rapidly and with minimal training by the clinical subjects themselves, if necessary. Clearly this offers real potential for serial sampling of subjects away from the clinic. As DBS results in a dry, lightweight sample, shipping and storage costs can be markedly reduced

compared with traditional whole blood sampling techniques that require frozen transportation and storage.

Analytically, the samples are very easy to handle. Samples for analysis are readily

obtained by punching a disc for analysis from the centre of the spot, followed by a simple liquid extraction of the punched sample. DBS samples show good recovery for the majority of analytes evaluated, with much cleaner extracts leading to improved chromatography and detection. Precision and accuracy of repeat spots has proven to be excellent, and many users are reporting improved sample stability, presumably resulting from the inactivation of enzymes in the sample and the fact that the analytes are no longer in solution.

With a sample as small as those obtained using DBS (typically 15µL), sensitivity can be a significant issue for LC-MS/MS assays. Lower doses, combined with low sample volumes, require highly sensitive assays to employ the technique. Automation with some miniaturisation of sample volume requirements via microplate techniques of extraction are now routine in many laboratories. However, this is still an area where sample volume usage remains relatively large when compared with the newer techniques in immunoassays. In response, multiple spotting techniques are being evaluated to balance blood volume and sensitivity limitations, and the introduction of increasingly sensitive LC-MS instrumentation, for example UPLC, are helping to address this challenge.

LIGAND BINDING ASSAYS

While in vogue, dried blood spotting is not the only approach available for conserving samples in clinical studies. There are a number of techniques currently being applied in the field of ligand binding assays that can offer significant reductions in the volumes of biological fluids required for each analysis. Multiplexing techniques allow multiple assays to be conducted in the same microwell. On the most mature platform (Luminex), this is done by labelling microbeads with different ratios of two dyes that can be detected and identified by flow cytometry, with selectivity being provided by molecule specific capture antibodies. Not only do such approaches reduce the sample volume required, they also improve

efficiency when compared to single analyte methods. It is possible to perform analysis for a large number of analytes on extremely small volumes (50µL for example). With the pros come some cons, including antibody cross reactivity and sensitivity issues for panels of markers with significantly different concentration ranges. However, multiplexing has established a clear role in early biomarker research where the objective is to evaluate a large group of markers to identify those that may be most beneficial to take forward into later stage clinical development. As a more discrete panel of markers is identified, the rigour of the assay can be improved to match the regulatory requirements for data submission. As a rule, it is recommended that no more than five analytes per multiplex in a validated quantitative assay are used. Other manufacturers and technologies have entered the Multiplex arena since Luminex led the way, and these include the Meso Scale Discovery and Searchlight platforms. All give great potential benefit in terms of sample volume requirements but each has its own specific limitations.

The advent of platforms using nanotechnology had provided additional opportunities for reducing sample size. The platform that has pioneered this for quantitative assays of macromolecules is the Gyrolab, manufactured by Gyros of Sweden. It is effectively a microfluidic immunoassay analyser and lends itself to pharmacokinetic, biomarker and immunogenicity assays for large molecules. This platform can compete with multiplex assays in the quantitative mode in terms of price but with the benefit of improved assay performance. Methods can be developed rapidly for individual analytes on a study specific basis, ensuring the right sensitivity and analytical range while avoiding the typical multiplex drawbacks of potential cross-reactivity, cross-talk, matrix interferences and similar analytical ranges. Up to five assays can be run simultaneously on the Gyrolab on the same samples – using as little as 10µL. Costs are reduced in two ways. Firstly, the labour element is significantly

reduced compared to more manual ligand binding techniques as the platform is fully automated. Secondly, the system uses a small fraction of the antibody required for standard 96 well microplate immunoassays, resulting in considerable savings in reagent costs.

There are many potential applications for these low volume techniques in clinical research. They are well suited to sampling in special populations who may be limited in their ability to attend clinics regularly, such as elderly subjects, or where sample volume is a particularly limiting issue. Given its early use in phenylketonuria, it is perhaps ironic that the wheel has almost turned full circle with techniques such as DBS now being looked at with renewed interest by drug developers in the paediatric arena. The increased regulatory focus on paediatric development plans has meant that being able to characterise pharmacokinetics in paediatric populations has assumed new importance. The well characterised advantages of low sample volume and the relatively non-invasive nature, coupled with more sophisticated pharmacokinetic techniques such as population pharmacokinetics, mean that DBS sampling is ideally suited to studies in this population.

THE POWER OF POP PK

Techniques such as DBS also have a promising application with regard to population pharmacokinetics (Pop PK), which seeks to identify the measurable pathophysiological factors that cause changes in the dose-concentration relationship, so that dosage can be appropriately modified if such changes are associated with clinically significant shifts in the therapeutic index. Patient demographic, pathophysiological and therapeutic factors, such as body weight, excretory and metabolic functions and the presence of other therapies, can frequently alter dose-concentration relationships (3,4). For example,

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steady-state concentrations of drugs eliminated mostly by hepatic metabolism are usually greater in patients suffering from degrees of hepatic impairment failure than they are in patients with normal hepatic function receiving the same drug dosage. The power of Pop PK relies upon collecting sparse samples from large numbers of subjects along with dosing details and covariates of interest, rather than taking extensive traditional sample profiles in a tiny subset of the total trial population. The ability to combine a home or clinic visit with a simple and reliable sampling technique such as DBS is self-evident and could significantly improve compliance and data quality in such studies.

Low sample volume techniques have also been applied to multiple analyte sampling for clinical biomarker studies. For example, Kapur *et al* recently reported using DBS to sample for cardiometabolic risk factors (5). While none of the risk factors in this study are accepted as surrogate endpoints, the ability to assess these and other analytes in clinical development can give useful supporting information which can assist internal decision making and Phase IV post-marketing surveillance. For any large multicentre clinical study, the ease of biological sample collection, storage and ambient transportation, coupled with the ability to extend the range of analytes that can be measured in any study, can have a significant impact on the cost and practicality of such evaluations.

CONCLUSION

There is a very real need to continue to use existing and new technologies better in order to further reduce sample volume requirements in the pharmaceutical industry, either to expand the data that can be acquired from any given study or to reduce the volumes taken from the subjects.

Various low volume analysis techniques can answer the sample use issue, but the ease of sampling and logistical advantages of DBS warrant the attention it has most recently garnered. While some low volume techniques may have advantages in sensitivity or offer greater facility for certain types of investigation, the selection of the appropriate technique is almost never simple. As ever, the available analytical technologies must be carefully matched to the scientific objectives of a clinical study to achieve an optimal outcome.

References

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