

What a drop of blood reveals

In blood analysis, precision, sensitivity and the required sample amount are important factors – along with the analysis time. In all these areas, Dried Blood Spot (DBS) sampling and analysis is a convincing alternative – especially when automated with the GERSTEL MultiPurpose Sampler (MPS).

By Oliver Lerch

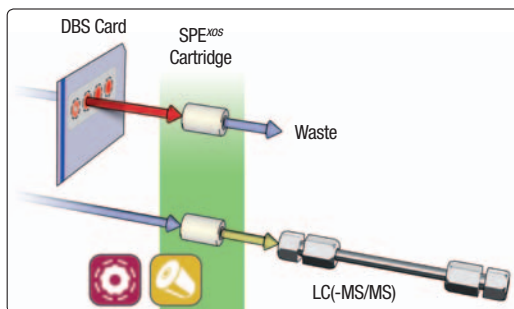
Since the 1960s, Dried Blood Spot (DBS) sampling has been used. Initially, especially neonatal screening for metabolic disorders was performed based on the DBS technique. In recent years, especially since more powerful GC/MS and LC/MS system became generally available, DBS has become useful in pharmaceutical research [1], forensic toxicology and doping analysis [2-4] as well as veterinary analysis [5].

Just a few drops of blood taken from a fingertip are sufficient to perform DBS analysis. The drops are placed onto small circular fields on special cards used for DBS work. As soon as the drops have dried, the cards are used to store and transport the samples, which are extracted directly from the card using dedicated sampling equipment.

A DBS sample typically contains between 15 and 30 μ Ls of blood evenly distributed across the spot. This means that a representative sample – and a defined amount of blood – can be taken by simply punching out a small area of the blood spot. Traditionally, a small, well defined disc of a few millimeters across has been punched out from the blood spot and transferred to a vial or micro-titer plate in which it was extracted using a suitable solvent. The resulting extract was centrifuged and the supernatant cleaned or analyzed directly following solvent exchange. Most often, LC-MS/MS or GC-MS/MS are the analysis techniques used. The described procedure is used for manual sample preparation based on DBS cards.

Automation improves productivity

In order to improve throughput and simplify the routine analysis workflow, the DBS technique must be properly automated. In cooperation with Spark Holland B.V., GERSTEL has developed an integrated system based on the Spark DBS system, with fully automated sampling from up to 240 DBS cards. Automation is performed using the GERSTEL MultiPurpose Sampler (MPS) under MAESTRO software control. The system can operate as stand-alone workstation preparing samples for LC/MS analysis - or integrated with the LC-MS/MS system. Slightly simplified, the DBS-MPS-LC-MS/MS System* works as follows: The MPS transports a DBS card to a camera. An image recognition software evaluates the dried blood spot. The



Schematic diagram of DBS flow-through desorption (FTD™) and SPE clean-up. FTD is patented by, and is a registered Trade Mark of, Spark Holland B.V.

card is loaded into the desorption interface and clamped into position. A desorption eluent then flows through a defined area of the blood spot desorbing the analytes based on the patented Flow Through Desorption technique (FTD™); an internal standard can be added to the desorption eluent for quality purposes if required.

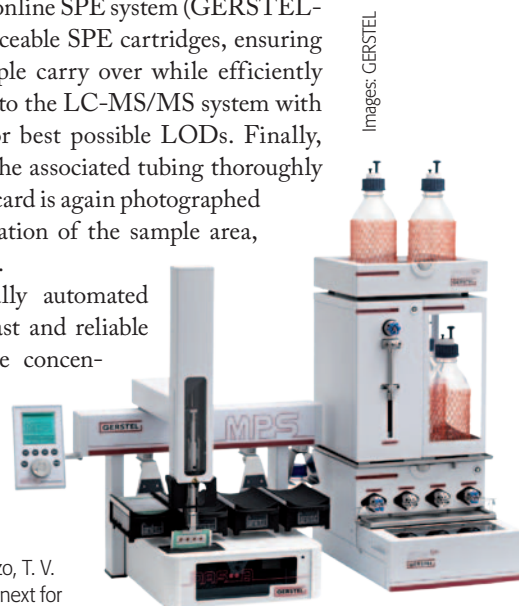
An eluate clean-up step can further be performed based on an

on-line SPE module which can be integrated into the overall analysis system. The online SPE system (GERSTEL-SPE^{nos}) is based on replaceable SPE cartridges, ensuring minimal sample to sample carry over while efficiently transferring the analytes to the LC-MS/MS system with best possible recovery for best possible LODs. Finally, the card is released and the associated tubing thoroughly rinsed with solvent. The card is again photographed to enable full documentation of the sample area, which has been desorbed.

Conclusion: The fully automated DBSA system enables fast and reliable determination of analyte concentrations with excellent LODs.

References

- [1] Q. C. Ji, G. Liu, C. J. D'Arienzo, T. V. Olah, M.E. Arnold, "What is next for dried blood spots?", *Bioanalysis* 4 (2012) 2059
- [2] C. P. Stove, A. S. Ingels, P. M. M. De Kesel, W. E. Lambert, "Dried Blood Spots in Toxicology: from the Cradle to the Grave?", *Critical Reviews in Toxicology* 42 (2012) 230
- [3] L. Tretzel, A. Thomas, T. Piper, M. Hedeland, H. Geyer, W. Schänzer, M. Thevis, "Fully automated determination of nicotine and its major metabolites in whole blood by means of a DBS online-SPE LC-HR-MS/MS approach for sports drug testing", *Journal of Pharmaceutical and Biomedical Analysis* 123 (2016) 132–140
- [4] L. Tretzel, C. Görgens, H. Geyer, A. Thomas, J. Dib, S. Guddat, V. Pop, W. Schänzer, M. Thevis, "Analyses of Meldonium (Mildronate) from Blood, Dried Blood Spots (DBS), and Urine Suggest Drug Incorporation into Erythrocytes", *International Journal of Sports Medicine*. DOI 10.1055/s-0036, <https://www.thieme-connect.com/products/ejournals/pdf/10.1055/s-0036-1582317.pdf>
- [5] F. D. Foster, J. R. Stuff, E. A. Pfannkoch "Automated Desorption, SPE Extraction, and LC-MS/MS Analysis of Dried Blood Spots", GERSTEL AppNote 4/2015



MPS-based GERSTEL DBSA Autosampler (to the left) with SPE^{nos}-Module (right hand side) for direct connection to the LC-MS/MS system (not shown).