FOCUS

Alturas Advisor

FALL WINTER 2009-10

Dried Blood Spot Analysis Coupled with LC/MS/MS

Dried blood spot (DBS) analysis has been receiving much attention as of late for use as a sample collection and analysis technique for the determination of pharmacokinetics of drugs for in-life and clinical studies (1). DBS is a technique where approximately 15 µL of blood is spotted on a punch card. The punch is removed from the card and extracted with a solvent. Thereafter, analysis may occur via LC/MS/MS or other methods. DBS has been used for routine testing in clinical labs for many years. However, DBS is a recent technique for bioanalysis to support large scale PK studies for in-life studies and clinical trials.

The technique has several advantages including, preferred sample collection for patients, small sample volume collections, typically prolonged sample stability, and ease of storage of samples. Patients prefer a "prick" of the finger rather than a venous sample collection. The small sample volume collection is advantageous for pediatric or small rodent studies where limited blood is available. Since blood spots are dried before storage or processing, sample stability is usually prolonged compared to a plasma, serum, or blood sample. Researchers have also shown that often ambient storage is sufficient for DBS programs, whereas for the same program -70 °C storage may be necessary in a plasma sample (2). Additional stability of DBS samples could be especially advantageous in a clinical trial where clinics in isolated areas of the world may not have adequate freezer capabilities.

As with any newly introduced technique to bioanalysis, the limitations of the technique must be discussed. Several limitations such as, decreased detectability due to small sample size, difficulty to automate punching of cards for sample analysis, inadequate precision and accuracy of method due to irreproducible blood spotting, and an apparent increase of the matrix effects with DBS coupled with LC/MS/MS are all issues that must be considered before widespread acceptance of the technique. For programs where detection of the components to <25 ng/mL is necessary, the limited blood volume (<15 µL) of DBS coupled with LC/MS/MS isn't an attractive alternative to conventional sampling where preconcentration can occur. Currently, instruments to handle punches and "solid" samples are not routinely available. However, development is on-going with vendors and researchers to improve the automation of DBS. Additional research is on-going to determine how to improve the reproducibility and accuracy of spotting the sample and reproducibly analyzing the DBS sample. The ESI/MS matrix effect issues can be solved with a better selection of extraction solvents and improvements in the selectivity of the LC separation.

The question remains, "will DBS be a routine tool accepted by bioanalysts, clinicians, toxicologists and

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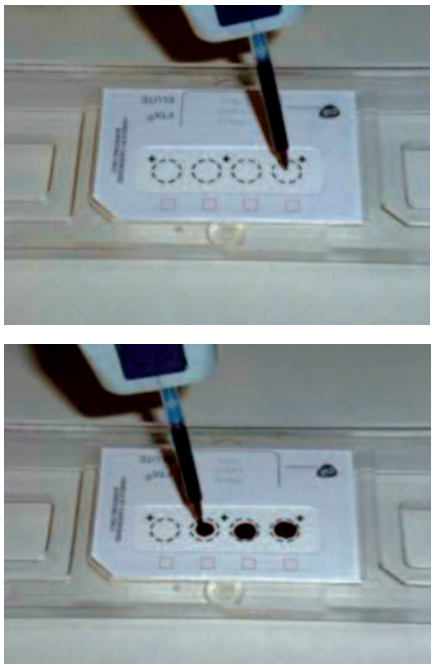
PK scientists to support drug development programs?" Alturas Analytics is in collaboration with our research partners to monitor the progress of this technique and be involved in its development and implementation. We look forward to watching DBS evolve. For more information regarding Alturas Analytics visit our website at *www.alturasanalytics.com*.

Blood spot card. Reproduced from *www.whatman.com*



Method of blood spotting. Reproduced from *www.whatman.com*

OUTREACH 2009-10

International Society for the Study of Xenobiotics (ISSX)-Poster Presentation: Validation of an HPLC/MS/MS Bioanalytical Method for the Quantitative Analysis of Lidocaine from Rat and Mini-Pig Plasma
October 18-22, 2009
Baltimore Marriott Waterfront
Baltimore, MD

Chemical and Pharmaceutical Structure Analysis (CPSA)-Short Course: "Method Development for LC/MS: Traditional Approaches and Emerging Trends"
October 26-29, 2009
Sheraton Bucks County Hotel
Langhorne, PA

American Association of Pharmaceutical Scientists (AAPS)-Exhibit
November 8-12, 2009
Los Angeles Convention Center
Los Angeles, CA

Pittcon-Short Course: "HPLC Methods Development for LC/MS"
February 28-March 5, 2010
Orange County Convention Center
Orlando, FL

49th Annual Society of Toxicology Meeting-Exhibit
March 7-11, 2010
Salt Palace Convention Center
Salt Lake City, UT

58th ASMS Conference on Mass Spectrometry-Exhibit
May 23-27, 2010
Salt Lake City, UT
Presentations Pending

11th Annual Land O'Lakes Bioanalytical Conference
July 19-23, 2010
Devil's Head Resort
Merrimac, WI

References:
1) N. Spooner et. al. Anal. Chem. (2009), 81(4), 1567.
2) N. Spooner et. al. 10th Annual Land O'Lakes Bioanalytical Conference Merrimac, WI (2009).

Qualification of Instruments in a Bioanalytical LC/MS/MS Lab: Evolution of Requirements

As instruments, software and technology continue to evolve, the guidance documents and regulations that govern the use of these technologies changes as well. To demonstrate an instrument can produce reliable and valid data in the pharmaceutical industry, users must qualify these instruments.

In 2006 the United States Pharmacopoeia (USP) published a new chapter <1058> on analytical instrument qualification, which was adopted in USP 30 in 2008. Generally, USP documents have been used in the GMP environment. However, especially with respect to analytical instruments and methods, there has been regulatory push to have common procedures in GMP and GLP laboratories. Consequently, GLP laboratories are using the USP <1058> as guidance for analytical instrument qualification.

Analytical instrument qualification (AIQ) is the phrase used for the process to ensure that an instrument is suitable for its intended purpose. In order to produce quality data an instrument must be qualified, then a method can be validated and the method(s) continually tested to ensure adequate performance. In this article we will focus on the discussion of only the component from the quality triangle of AIQ discussed in USP <1058>. See USP <1058> for a more in-depth discussion of the quality triangle.

Activities for qualification of instruments can be grouped into four phases, each performed in the following order: design qualification, installation qualification, operational qualification, and performance qualification.

Design Qualification (DQ) is the documentation that describes the specifications of the instrument and criteria for selection of the vendor and user requirements for the instrument. DQ may be performed by the instrument vendor and/or the end user. The manufacturer is responsible for the design and maintenance of documents that describe how the instrument is manufactured and tested before delivery to end users. Nevertheless, end users must ensure the instruments are suitable for their intended application and that the vendor has quality systems in place to provide reliable equipment.

Installation Qualification (IQ) is the documentation that describes that an instrument is delivered as designed, and is properly installed in a suitable environment per the user requirements. IQ is necessary for new, used, or any previously unqualified instrument.

Operational Qualification (OQ) is the documentation that describes how the instrument functions according to specification in the newly installed environment. Testing activities against user requirements in the OQ phase may include, fixed parameters (e.g. acceptable height, weight, length and voltage inputs), secure data storage, backup, archiving, audit trails, and instrument function tests (e.g. voltage outputs).

Performance Qualification (PQ) is the documentation that describes how the instrument performs according to user requirements in the newly installed environment. PQ is a series of routine analytical tests using known components, which may include standards or quality control samples during routine analysis. PQ tests should demonstrate that the instrument is operating and performing as required by the end user. If an instrument fails to meet the specifications of PQ testing, the instrument requires repair, maintenance, or calibration.

The attraction of Chapter 1058 in the USP is that the procedures described within are based on sound science in consideration of regulatory oversight. This means that as long as the qualification approaches can be scientifically supported and documented per SOP, the FDA will be reasonable in its evaluation and acceptance of the data generated. Alturas Analytics, Inc. has an SOP, along with supporting documentation, to address all the needs of AIQ. For more information regarding Alturas Analytics visit our website at *www.alturasanalytics.com*.

STAFF PROFILE: Casey Johnson

Casey Johnson is an Associate Scientist at Alturas Analytics, Inc. His responsibilities include LC/MS/MS method development, validation, and sample analysis for small molecule drugs and peptides.

Casey came to work for Alturas Analytics after receiving a Bachelors of Science from the University of Idaho, where he performed undergraduate research on zinc transport in yeast cells. Casey started his career at Alturas nearly three years ago as a lab assistant. With training and experience Casey became well versed in LC/MS/MS and bioanalysis. Now as an Alturas scientist, he works on a wide variety of projects in support of both GLP and non-GLP studies.

Casey lives in Moscow, Idaho, with his wife Kim. He enjoys backpacking and fly fishing.

The LC/MS Experts™

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