

Alturas Advisor

FALL WINTER 2010-11

INSIDE THIS ISSUE

DISCUSSION CORNER PAGE 2

OUTREACH PAGE 3

STAFF PROFILE PAGE 4

Research Advances with Dried Blood Spot Analysis (DBS) Coupled with LC/MS/MS

In our newsletter last year, we posed the question, "Will dried blood spot (DBS) analysis become a routine tool accepted by bioanalysts, clinicians, toxicologists and PK scientists to support drug development programs?" Although the technique is still in its infancy in the pharmaceutical industry, the past year has seen DBS technology emerge into an accepted bioanalytical tool. Many organizations, including AAPS and ASMS, have devoted entire conference sessions to the technique and plan to include sessions in their upcoming meetings. Interest continues to grow, and we are proud to say that Alturas Analytics is

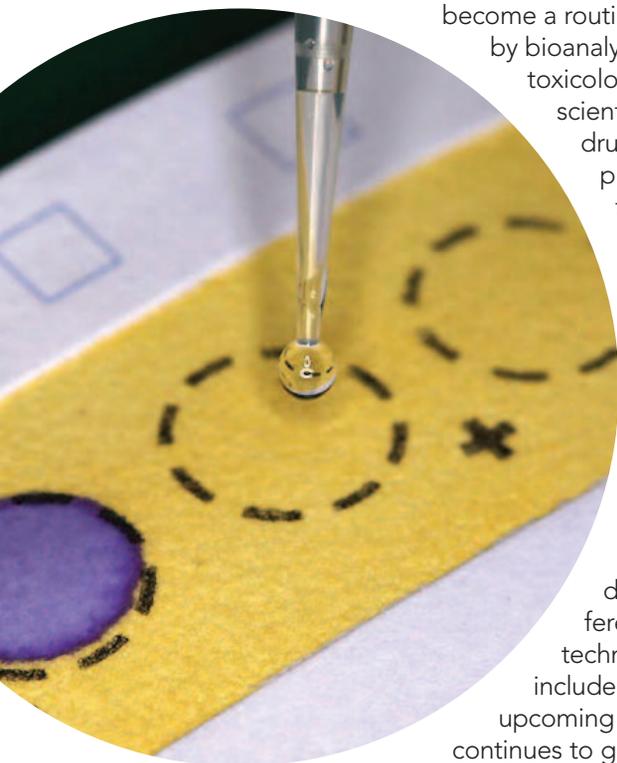


Figure 1.

one of the leaders in this exciting bioanalytical research field.

As the DBS technique and technology become more widespread, materials and methods are developing to accommodate FDA guidelines and ease arduous sample processing requirements. Advances in collection card materials allow for better recovery and reduced matrix effects. Additionally, techniques that will allow for automated punching of cards promise to reduce sample preparation time. "Stacking" methods (combining multiple punches in one extraction vessel) continue to improve the limits of detection for DBS. Using stacking, Alturas Analytics has developed DBS methods in conjunction with LC/MS/MS with LLOQs in the pg/mL range. Applications of DBS are also being developed to support ligand binding assays of biomolecules, proteins and biomarkers. Advancements continue as other vendors enter the market to create novel DBS collection paper and cards.

Much of the early DBS work involved analysis of drugs and metabolites for DMPK assessments in whole blood. Based on discussions with sponsors and their needs for collection and analysis of translucent and often limited volume fluids (CSF, synovial fluid, tears, etc.), Alturas Analytics recognized the possibility of applying DBS techniques to the analysis of fluids other than whole blood. The scientists at Alturas Analytics have developed bioanalytical methods in tears, synovial fluid, urine, plasma, CSF and saliva using a small volume of sample (5-30 μ L) spotted onto collection paper. Since the matrix is not limited to whole blood, the term Dried Matrix Spot (DMS) has been created to more accurately reflect the process.

Although the DMS technique is identical to DBS, unique challenges have been identified when DMS is used with translucent fluids. Once dried, the DMS

(continued on page 2)

Bioanalytical Harmonization across the Globe

Bioanalytical support of new drug development and bioequivalents now takes place across the globe. As sponsors seek to distribute new or bioequivalent drugs to emerging and mature markets worldwide, regulatory filings, and the bioanalysis needed to support them, occur in the Americas, Asia, Europe, the Middle East and Africa. While the majority of bioanalytical guidelines are similar across the globe, different regulatory environments often have different criteria for what is considered acceptable for method validation and sample analysis. Data generated for one regulatory environment might not be considered acceptable in another, which can lead to misunderstandings and potential delays in the application process or approval of a drug.

To help resolve this problem leaders in the bioanalytical industry have assembled a Global Bioanalysis Consortium (GBC). The GBC will have representatives and input from the pharmaceutical industry, CROs, academia and regulatory agencies from across the globe. The goal of the GBC will be to harmonize global bioanalytical guidance for method validation and sample analysis. The leaders of the GBC recognize the challenges involved in undertaking such a global harmonization. However, they realize this global effort will eventually pay off in the form of more efficient and timely future drug applications. The FDA has stated a desire and willingness to work with the EMA and other agencies to facilitate such global bioanalytical guidance.

The vision of the GBC has already been presented at several conferences and meetings. The annual AAPS meeting will be a major venue where the GBC will provide updates on advancements in harmonization. As a bioanalytical CRO that works with sponsors worldwide, we applaud the efforts of the GBC and are excited about the harmonization of global bioanalytical guidance. Alturas Analytics will continue to monitor the progress of the GBC to determine the effect such guidance will have on our collaborations with sponsors. For more information regarding Alturas Analytics, please visit our website at www.alturasanalytics.com.



(continued from page 1)

is clear and the exact location of the spot cannot accurately be determined visually, making it difficult to know exactly where to punch out the sample spot. In order to overcome this challenge, Alturas Analytics has developed a color-indicating technique that can be used to determine the exact sample spot location. A color-indicating dye is added to the collection paper and then allowed to dry. Once dry, the sample can be spotted onto the collection paper and the exact spot location can easily be determined prior to removing the sample punch. See Figure 1.

With DMS technology that allows the visual detection of clear matrix fluids, the applications for DMS expand considerably. DMS analysis could also be applied to plasma, particularly if historical data was not collected using whole blood. Plasma DMS removes the need for a bridging experiment if the compartment for PK analysis is changed from plasma to whole blood. We have found DMS advantageous in other matrices as well, including urine and in-vitro fluids. For these highly aqueous fluids, DMS typically gives less non-specific binding than the traditional vial, tube or plate collection methods.

Alturas Analytics has developed and validated DMS methods that follow the latest bioanalytical guidance and white papers. A recent example is the determination of dexamethasone from pig synovial fluid. See Figure 2 and Table 1. The method was selective, accurate and precise. A manuscript based on this method was accepted for publication in an issue of Bioanalysis devoted to DBS (November 2010).

Based on the exciting developments and meaningful data generated in the past year with DBS/DMS technique, it appears that DBS/DMS is here to stay. DBS/DMS analysis will continue to evolve and

provide the pharmaceutical industry with another tool for bioanalytical measurements. Research in DBS and DMS continues at Alturas Analytics and we look forward to our continued collaboration with vendors and sponsors as we extend the uses of this technology. For more information regarding DMS and other bioanalytical assays at Alturas Analytics, please visit our website at www.alturasanalytics.com.

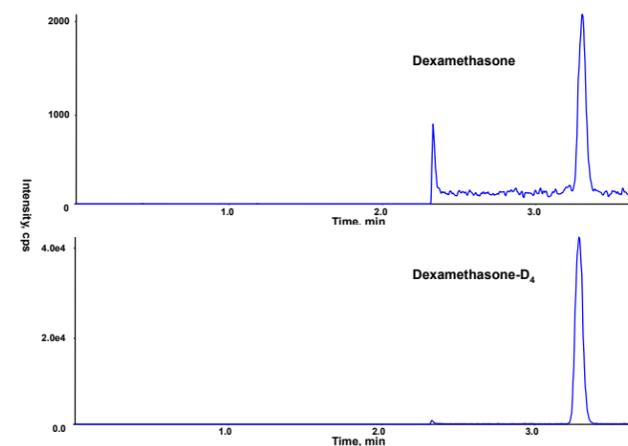


Figure 2. HPLC/MS/MS Chromatogram of Dexamethasone Extracted from Pig Synovial Fluid at 5.00 ng/mL

Table 1. Interassay Accuracy and Precision for the Determination of Dexamethasone from Pig Synovial Fluid Using DMS

QC Concentration (ng/mL)				
Accuracy in %±CV, 3 days, n=18				
5	15	50	400	500
102.6	99.6	101.2	96.1	100.7
±14.2	±7.8	±10.0	±7.7	±9.7

OUTREACH 2010-11

Chemical and Pharmaceutical Structure Analysis (CPSA)
Short Course: Method Development for LC/MS: Traditional Approaches and Emerging Trends
Poster and Oral Presentations
October 18-21, 2010
 Sheraton Bucks County
 Langhorne, PA

American Association of Pharmaceutical Scientists (AAPS)
Exhibit and Poster Presentation
November 14-18, 2010
 Morial Convention Center
 New Orleans, LA

Society of Technology (SOT)
50th Annual Meeting
March 7-11, 2011
 Walter E. Washington Convention Center
 Washington, DC

Pittcon
Short Courses: "Development and Validation of Dried Blood Spot Analysis Methods in the Bioanalytical Laboratory" and "HPLC Methods Development for LC/MS"
March 13-18, 2011
 Georgia World Congress Center
 Atlanta, GA

Canadian LC-MS Group (formerly CVG)
The 5th Workshop on Recent Issues in Regulated Bioanalysis
April 11-14, 2011
 Montreal, ON

AAPS 2011 National Biotechnology Conference
Co-Chairing Session on "Dried Blood Spot Analysis for Biopharmaceuticals and Biomarkers"
May 16-18, 2011
 Hilton San Francisco Union Square
 San Francisco, CA

59th ASMS Conference on Mass Spectrometry
Presentations Pending, Exhibit
June 5-9, 2011
 Denver, CO

12th Annual Land O'Lakes Bioanalytical Conference
July 2011
 Devil's Head Resort
 Merrimac, WI



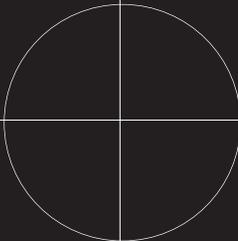
STAFF PROFILE: Chrystal Sheaff

Chrystal Sheaff is a Scientist at Alturas Analytics, Inc., where she has been a member of the Alturas family since 2008. Chrystal performs bioanalysis on small molecules using LC/MS/MS. She is involved in many facets of the laboratory including method development, assay validations, and sample analysis. Chrystal is also actively performing research on the use of Dried Matrix Spot (DMS) technology for analysis of compounds in clear fluids. In addition to her laboratory responsibilities, Chrystal is actively involved in writing test methods, drafting technical procedures, and editing standard operation procedures (SOPs).

Chrystal has a Ph.D. in Chemistry from the University of Idaho and a Bachelors of Science degree from Black Hills State University, where she majored in biology and chemistry. As an undergraduate she worked in Dr. D. Bergmann's laboratory where she used genomic DNA and polymerase chain reaction (PCR) in her research. Chrystal's

graduate research, in Dr. Chien M. Wai's laboratory, focused on the reduction products of nitroaromatic vapors commonly found in explosives. Several catalysts, including carbon nanotube supported-metal nanoparticles were used for the catalytic hydrogenation of the vapors. After reduction, several fluorescence spectroscopy techniques including synchronous, derivative, and excitation-emission matrices (EEMs) were used for detection.

Chrystal lives in Moscow, ID, with her husband John, who teaches physical education and coaches football for the Moscow School District. As with most South Dakota natives, Chrystal loves motorcycles, barbequing, and Chislic (cubed meat, deep-fried). Since living in the Inland Northwest she has enjoyed touring on her Harley Davidson, attending high school and collegiate sporting events, and welcoming daughter Brooklyn to her family.



The LC/MS Experts™

www.alturasanalytics.com

TOLL FREE: 877.344.1279 • PHONE: 208.883.3400 • FAX: 208.882.9246