FOCUS

Alturas Advisor

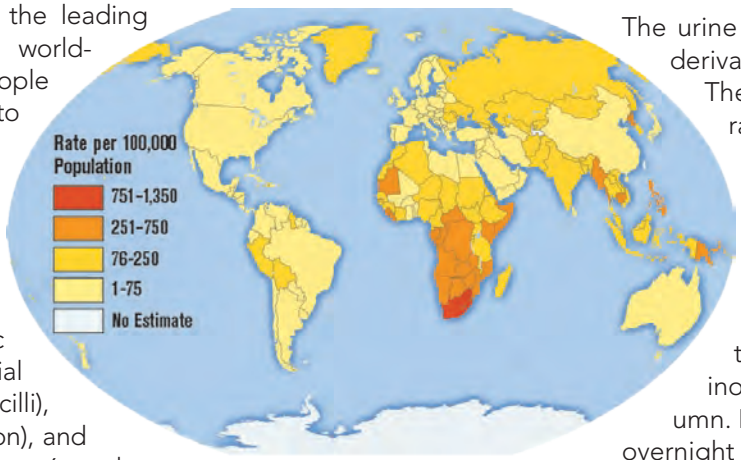
FALL WINTER 2016-17

A Better Diagnostic Test for Tuberculosis: GC-MS/MS Bioanalysis for the Determination of the Biomarker D-Arabinose in Urine

A collaborative article with
The Global Good Fund and Alturas Analytics, Inc.

Alturas Analytics, Inc. excels in the ability to solve problems and meet the 21st century's demands in the treatment of disease. The future of bioanalysis is the convergence of drug development with clinical diagnostics (biomarkers) and we remain dedicated to the managed expansion of our throughput capacity and the variety of services offered in our laboratory.

Tuberculosis is a global pandemic found in every country in the world and is the leading cause of infectious death worldwide. Close to 10 million people worldwide are estimated to have fallen ill with TB in 2014. Diagnosis of pulmonary TB is challenging and involves clinical assumptions with abnormal chest x-rays and analysis of sputum for the TB bacteria. Current diagnostic testing relies on differential stains for AFB (acid-fast bacilli), NAA (nucleic acid amplification), and AFB cultures that can take up to 6 weeks from multiple sputum samples. GC–MS is a very different technique with significant potential for further development to an accurate, sensitive and specific technique for the confirmation of TB in urine samples. Urine facilitates ease of sample collection compared to sputum.



Over the last year Alturas Analytics, Inc. has utilized gas chromatography mass spectrometry (GC-MS) and gas chromatography-tandem mass spectrometry (GC-MS/MS) for the analysis of molecules that are challenging for traditional liquid chromatography and LC-MS. One application is the analysis of the biomarker D-arabinose from urine for confirmation of the diagnosis of TB.

The cell wall of tuberculosis is unique and consists of a variety of lipids, lipoglycans, polysaccharides, fatty acids and other components. One of the principle components of the cell wall of the mycobacteria is lipoarabinomannan (LAM). Previous research indicates that D-arabinose is a surrogate for LAM and thus can be used as a surrogate to detect an infection of tuberculosis. (2). D-arabinose is a small, polar molecule that is not favorable for ionization with LC-MS and is not easily retained on conventional HPLC columns. Other methods to detect LAM such as immunoassays suffer from poor sensitivity or laborious steps of sample preparation.

The urine samples were extracted and derivatized to improve separation. Then, the aliquots were evaporated until no liquid remained. The samples were reconstituted in an organic solvent and injected onto one of our Thermo Scientific's TSQ Quantum XLS Ultra™ GC-MS/MS systems. To separate the D-arabinose from L-arabinose, we used a chiral GC column. Previous methods required an overnight derivatization process using chiral reagents to distinguish between the D- and L- forms of arabinose.

The method for the GC-MS/MS analysis of D-arabinose will be effective for the confirmation of TB and improves upon previous methods that required a number of steps and more materials and reagents. This method will help

(continued on page 2)

STAFF PROFILE:

Rachel Walker

Rachel Walker is an integral part of the Quality Assurance team at Alturas Analytics, Inc. Her focus on improvement of quality systems, attention to detail, and constructive attitude provides confidence that data delivered to our clients meets the highest standards of quality and integrity.

Rachel joined Alturas Analytics, Inc. in the summer of 2011 as Sample Custodian, and has since held positions as Technical Writer, Archivist, and Standard Operating Procedure (SOP) Manager before entering the Quality Assurance department in 2013. Rachel's experience in various roles within the company continues to be an asset when conducting study and facility based inspections, giving her insight on potential improvements to the accuracy and efficiency of our processes. She currently audits data and study-based activities, participates in facility inspections, and assists in developing Quality Assurance SOPs, technical procedures, and forms.

Rachel is a north Idaho native from the nearby city of Lewiston. She relocated to Moscow in 2006 and completed bachelor's degrees in Microbiology and Virtual Technology and Design from the University of Idaho. Rachel loves the natural beauty of the Palouse area and enjoys walking on scenic pathways, gardening at her home, and working on painting and sculpture projects. She is also a big fan of Mariners baseball and Washington State University football, cheering for her teams each season.

HPLC Column Technology in a Bioanalytical CRO

Ryan Collins, Shane Needham
LC/GC Magazine - Chromatographyonline.com, Apr 01, 2016, Special Issues, Volume 34, Issue 4, pg 24–27

Over the course of the last several decades, high pressure liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) has become the method of choice for high throughput analysis of small molecule therapeutics, metabolites and biomarkers. This is due in large part to the selectivity and sensitivity provided by HPLC-MS/MS, combined with the ability to rapidly develop an assay comprised of quick extractions and short run times for a vast majority of small molecules.

One way to increase HPLC-MS/MS productivity is to decrease column packing particle size. By decreasing the particle size below the previous standards to sub-2 µm particles, there is an increase in chromatographic efficiency (1). One of the side effects of decreasing the particle size is a large increase in pressure. In order to withstand the backpressures involved, new instrumentation was devised, and thus, what has been dubbed ultra-high pressure liquid chromatography (UHPLC) was born (2, 3). Using a UHPLC system, it is possible to successfully implement smaller particle size columns and run at pressures up to ~20,000 psi (4). These UHPLC systems have proven to be robust enough for high throughput bioanalysis work and have been implemented throughout the industry.

A recent advent to the column market in the last decade is superficially porous particle (SPP) columns. Rather than decreasing the size of fully porous particles in the columns, the idea behind SPP is a small, solid inner core surrounded by a permeable shell of porous silica. While the outer shell of the particles are similar in materials and function as a conventional fully porous column particle, the inner core is impermeable (hence the term superficially porous). With the combination of the small diameter of the inner core and the porous nature of the shell, SPP provides the benefits of sub-2 µm fully porous particle columns while eliminating much of the backpressure issues (6, 7). This increased rate of diffusion provides more efficient separations than was previously possible with fully porous columns. (8)

A Better Diagnostic Test for Tuberculosis:

(continued from page 1)

diagnose TB and deliver more cost effective and rapid treatments to communities around the globe.

Our team brings the experience and knowledge you need to address your most demanding bioanalytical challenges with the outstanding customer care and attention to detail that our clients have come to expect. Please visit us online at www.alturasanalytics.com or call 208-883-3400 for more information regarding our services, assays and research initiatives. Whatever your bioanalytical needs, we look forward to providing you with excellence.

1. World Health Organization Factsheet. June 25, 2016. who.int/tb/publications/factsheet_global.pdf?ua=1
2. Prithwiraj De, Anita G. Amin, Eloise Valli, Mark D. Perkins, Michael McNeil, Delphi Chatterjee, Estimation of D-Arabinose by Gas Chromatography/Mass Spectrometry as Surrogate for Mycobacterial Lipoarabinomannan in Human Urine. PLoS One. (2015) 10(12).

Emerging Technologies

Yet another approach of increasing efficiency in bioanalytical analysis is the implementation of microflow LC (MFLC) coupled with a mass spectrometer. MFLC-MS/MS employs the use of pumps that can accurately deliver a flowrate of well below 100 µL/min. The reduction in solvent use directly translates to a cost savings. The drastically lower flowrates associated with MFLC-MS/MS also translate to less solvent flowing through the ESI source. This means a cleaner MS and less cost associated with MS maintenance.

In terms of columns, MFLC-MS/MS employs the use of columns with drastically decreased internal column diameter (ID). While standard HPLC-MS/MS may use columns with IDs ranging from 2 – 4.6 mm, MFLC-MS/MS utilizes columns ranging from 0.2 – 0.3 mm (micro) down to < 0.2 mm (nano), which can run at 10 and 0.3 µL/min, respectively. Solvent consumption and savings aside, MFLC-MS/MS has also been documented to increase ESI response (9) while reducing matrix effects (10) and increasing ionization efficiency (11). Early work on ESI response demonstrated that as the mobile phase flow rate of ESI is reduced, there is an increase in proportional MS signal-to-noise ratio (12).

Some of the challenges in the integration of MFLC-MS/MS into the high throughput bioanalysis world are longer run times and dead volumes in fittings/connections having a greater impact on chromatography. There is also a perceived lack of robustness of microflow instrumentation. By integrating a column directly into the source, many of the dead volume issues related to MFLC-MS/MS are resolved. The idea behind the application is to simplify instrument set-up by minimizing connections and reducing the length of tubing required between the LC injector and the MS, and thus minimizing the impact of pre-column and post-column volumes.

Conclusion

With the advancements in column and other LC related technology in recent years, developing robust methods for novel therapeutics has become a more reliable process than ever. It is possible to efficiently create productive methods for molecules of ever-increasing complexity. This will become more important in years to come as HPLC-MS/MS is increasingly looked to as the solution for analysis of large molecules including peptides, proteins, and biomarkers. Looking to the future, the expectation for the pharmaceutical and biotech industries will be to supply the global community with therapeutics at a reasonable cost. Thus, the highest levels of productivity and efficiency will be paramount to meet this goal.

1. J.E. MacNair, K.C. Lewis, J.W. Jorgenson, Anal. Chem. 69, 983–989 (1997).
2. J.E. MacNair, K.D. Patel, J.W. Jorgenson, Anal. Chem. 71, 700–708 (1999).
3. N. Wu, J.A. Lippert, M.L. Lee, J. Chromatogr. A 911, 1–12 (2001).
4. In the news, Trends Anal. Chem. 61, iv–x (2014).
5. C. Horváth, B.A. Preiss, S.R. Lipsky, Anal. Chem. 39, (1967) 1422.
6. J.J. DeStefano, T.J. Langlois, J.J Kirkland, J. Chrom. Sci. 46, 254-260 (2008).
7. D. V. McCalley, J. Chromatogr. A 1218, 2887−2897 (2011).
8. G. Guiochon, F. Gritti, J. Chromatogr. A 1218, 1915-1938 (2011).
9. G. Valaskovic, N. Kelleher, Curr. Top. Med. Chem. 2(1), 1–12 (2002).
10. E. Gang, M. Annan, N. Spooner, P. Vouros, Anal. Chem. 73(23), 5635–5644 (2001).
11. R. Juraschek, T. Dulcks, M. Karas, J. Am. Soc. Mass Spectrom. 10, 300–308 (1999).
12. P. Kebarle, L. Tang, Anal. Chem. 65, 972A–986A (1993).

Outreach 2016-17

12th Annual Applied Pharmaceutical Analysis (APA)
Silver Sponsor
September 12-14, 2016
Boston Marriott, Burlington, MA

Clinical & Pharmaceutical Solutions through Analysis (CPSA) USA
Analytical Chemistry at a Crossroad: Making Data Relevant to the Patient
September 26-29, 2016
Sheraton Bucks County Hotel, Langhorne, PA

American Association of Pharmaceutical Scientists (AAPS) Annual Meeting & Exposition
Exhibiting - Booth #1736
November 13-17, 2016
Colorado Convention Center, Denver, CO

56th Annual Meeting and ToxExpo - Society of Toxicology
Attending
March 12-16, 2017
Baltimore, MD

American Society for Clinical Pharmacology and Therapeutics (ASCPT)
Attending
March 15-18, 2017
Washington Marriott Wardman Park, Washington, DC

11th Workshop on Recent Issues in Bioanalysis (WRIB)
Silver Sponsor
April 3-7, 2017
Hilton Los Angeles/Universal City, Los Angeles, CA

AAPS National Biotechnology Conference (NBC)
Exhibiting
May 1-3, 2017
San Diego Marriott Marquis and Marina, San Diego, CA

65th ASMS Conference on Mass Spectrometry and Allied Topics
Presenting
June 4-8, 2017
Indianapolis, IN

STATE OF THE COMPANY 2016

The Alturas Advisor newsletter began in 2002, and since then it has become an annual tradition. This year it heralds our 16th year in business and our commitment to push the boundaries of bioanalytical methods and applications in MS/MS. We have flourished in 2016. In this letter we will share the key to our growth and our plans to continue to provide our clients with high quality bioanalytical results in a timely manner.

First, we want to give a huge Thank You to the people who give us our purpose:

- To our clients for giving us the opportunity to accelerate therapeutic breakthroughs with quality science
- To our team for their dedication and commitment to personalized customer service
- To the many friends, vendors, family, and peers whose wisdom and cooperation are an important part of our success

LC-MS/MS and GC-MS/MS technologies are always improving to meet 21st century demands in the treatment of disease. To meet these demands, we remain dedicated to the managed expansion of our throughput capacity and the variety of services offered in our laboratory to better serve our clients and fulfill our mission to be the MS/MS Bioanalytical Experts.

Since opening in 2000 with one instrument, two people, and a vision to create an enduring company, Alturas Analytics has anticipated the needs of our clients and successfully managed growth. This year has seen new investment in capital equipment and scientific staff resulting in increased capacity of 25%, and completion of a fully functional laboratory that increased our operating space by 30%. We hit a record of 60% increase in team hires in 2016. To manage workflow forecasting, we have implemented software solutions focused on project management that yield shorter times to data and final reports with a great degree of confidence.

Perhaps our most impressive accomplishment of the year is our support of over 20 local and national non-profit groups including Christmas For Kids, the National MS Society, the Leukemia and Lymphoma Society, the ALS Association, Gritman Medical Center, the Palouse Discovery Science Center, and many area youth sports organizations.

At Alturas Analytics, we strive to increase our value to our clients every day. We pride ourselves on delivering timely bioanalytical results with the highest level of integrity. We anticipate many more years of success and look forward to continuing to work with our clients on breakthrough therapies to cure disease and enhance life.

Thank you for an abundant year!

Sincerely,
The Alturas Team

The LC/MS Experts™

www.alturasanalytics.com

PHONE: 208.883.3400 • FAX: 208.882.9246