Alturas Advisor
FALL WINTER 2013-14

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Driving Innovation in Regulated Bioanalysis through Microflow Liquid Chromatography and High Flow Nanospray Mass Spectrometry

Research continues at Alturas Analytics into Microflow Liquid Chromatography (MFLC) for its application in a regulated bioanalytical laboratory. As the technology improves and we refine our techniques, the advantages of this system become more self-evident. Last year the Alturas team successfully validated an assay for the analysis of methotrexate in plasma using MFLC-MS/MS. Since then our team has conducted numerous experiments and presented the use of MFLC-MS/MS under several conditions, in varying matrices and for differing purposes.

The benefits of low-flow analytical techniques are many and have been generally acknowledged for quite some time. These benefits include a higher ESI response [1], reduced matrix effects [2] and reduced solvent consumption [3]. As research expands the perceived limitations of MFLC, namely ruggedness over time and ease of use, are being addressed and overcome.

In the last year Alturas scientists have shown that MFLC-MS/MS is a useful analytical technique for large biological molecules, especially when limited solvent consumption and sample volume are a factor. Trypsin digestion was used for three proteins – myoglobin from horse skeletal muscle, human somatotropin and human ceruloplasmin in buffer solution. In order to show the relationship between flow rate and instrument signal, analysis of one tryptic digestion was also performed using three column diameters with flow rates ranging from 10-400 µL/min, according to column diameter. The resulting data showed that under the same instrumental conditions MFLC-MS/MS saw an increase in sensitivity between 3 and 5 fold. Furthermore, using the variable column diameters and flow rates we were able to achieve a sensitivity gain of more than 12 fold using 10 µL/min and 400 µL/min, respectively.

Another interesting discovery was made during the methotrexate validation (i.e. HPLC-MS/MS vs. MFLC-MS/MS). Throughout the validation we decided to record source contamination of both of the validation methods. Since only ~124 mL of solvent was needed to perform the MFLC-MS/MS validation compared to ~2500mL that conventional HPLC-MS/MS requires, it's possible that the lower volume was responsible for the significant decrease in the contamination of the source (image 1, image 2).

While recent work has continued to demonstrate the benefits of MFLC-MS/MS, our latest and most exciting research has been combining MFLC-MS/MS with a novel in-source LC column called PicoFuze™. In collaboration with New Objective, Inc., Alturas Analytics has been testing this first in class technology. PicoFuze™ consists of a modified MS source probe containing an integral LC column and nanospray emitter (figure 1). In this manner, "the column is the source" and "the source is the column". Thus fewer connections are needed to introduce ions into the MS. The reduced flow and

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STAFF PROFILE:
Staci Loughney

Staci Loughney joined the staff of Alturas Analytics in 2009. Her work ethic and attention to detail made her a perfect fit for the Quality Assurance Unit (QAU). As Quality Assurance Assistant she is responsible for aiding in the evaluation, maintenance and update of Alturas Analytics SOPs and regulations.

In carrying out those duties Staci regularly performs inspections and audits at critical phases of a study. This includes inspection of protocols, in-process activities, test methods and a variety of reports. She also maintains various databases and assists with sponsor audits of Alturas facilities. Staci has completed two courses/workshops at the West Coast Quality Training Institute. She has been a member of the Society of Quality Assurance since 2009. Over the years Staci has completed a number of webinars that cover a wide range of issues pertaining to GLP compliance.

Staci graduated from Washington State University with a Bachelor of Arts in Communication. In her free time Staci takes advantage of living in the Pacific Northwest. She enjoys hiking and camping in the Cascade Mountains and Mt. Rainier with her husband Brent, daughter Ainsley and dog Mandi. Staci is also a film buff, and favorites include Gone with the Wind, Goonies and Poltergeist.

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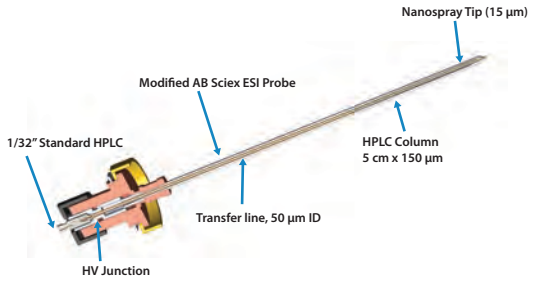
Figure 1 **PicoFuze™ Schematic**



minimized connections will provide optimal MS signal and improve peak shape.

For initial experiments, an AB SCIEX 5500 QTRAP mass spectrometer was fitted with a PicoFuze™ probe and coupled to an Eksigent ekspert microLC 200 microflow system. With no method optimization except lowering flow rate, temperature, and ESI voltage, methotrexate extracted from plasma was detected quantitatively. To then demonstrate ruggedness more than 65 methotrexate sample injections were performed. Again, with minimal method optimization there was no change in peak shape, signal, or retention time, showing promise for the application of PicoFuze™ for high throughput bioanalysis.

Our next step was to compare PicoFuze™ to not only conventional HPLC-MS/MS, but standalone MFLC-/MS/MS as well. Analysis of the digestion of human monoamine oxidase B (MAOB) was used to compare the sensitivity of the three analytical techniques. The same stationary phase (C18) and column length (50mm) were used for all three techniques. Column diameter and flow rates were scaled down appropriately to provide a direct comparison of instrument signal. Column IDs and flow rates for traditional HPLC, MFLC, and PicoFuze™ were 2.0 mm/700 µL/min, 0.50 mm/44 µL/min, and 0.20 mm/7 µL/min, respectively. The resulting data (figure 2) shows that not only is PicoFuze™ comparable with MFLC-MS/MS, but it provides greater signal even right out of the box. With more optimization and development we expect to see even greater gains in instrument signal using PicoFuze™.

As Alturas scientists continue experiments with PicoFuze™ we are finding that due to a significant increase in ionization efficiency signal increases considerably. This integration of column and source also means less fittings and connections, which reduce the risk of instrumentation downtime. The result is a "plug and play" approach to LC/MS and the ease of use increases efficiency and productivity. The reduced injection volumes needed to obtain adequate signal with PicoFuze™ also gives the analyst piece of mind knowing that many injections are possible from the same sample if reanalysis is necessary.

To bolster research even further Alturas Analytics recently purchased an AB Sciex 6500. We're excited to see the results of the 10X increase in sensitivity of the 6500 over the 5500 combined with

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PicoFuze™ MFLC technology. As the industry and regulations change more will be demanded of the bioanalytical laboratory. In collaboration with our sponsors and vendors we are excited to continually push the boundaries of what's possible in LC-MS/MS bioanalysis. For more information about Alturas Analytics' assays, research and services please visit us at www.alturasanalytics.com.

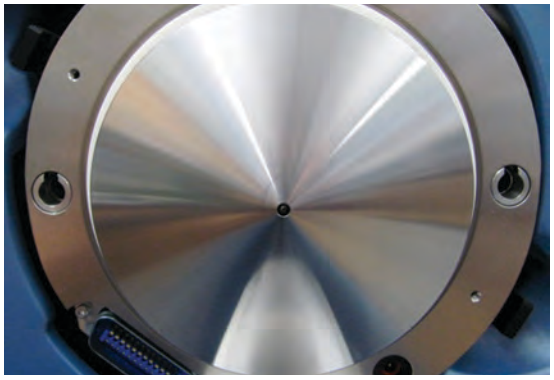


Image 1: QTRAP® 5500 interface plate after ~400 extracted human plasma injections from the Eksigent system – no switching valve

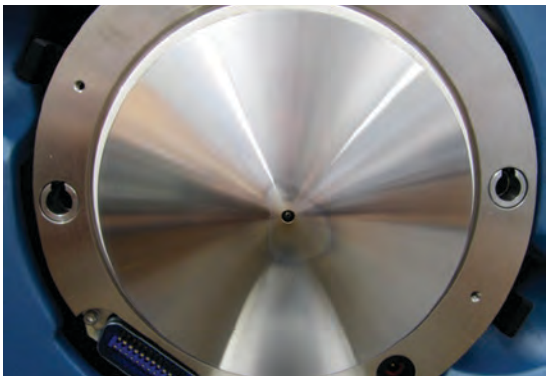
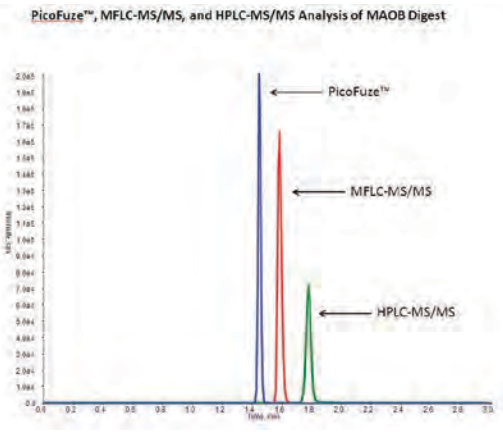


Image 2: QTRAP® 5500 interface plate after ~150 extracted human plasma injections from the conventional HPLC system – no switching valve

Figure 2



References:
1) Valaskovic G, Kelleher N. Miniaturized Formats for Efficient Mass Spectrometry-Based Proteomics and Therapeutics Development; Current Topics in Medicinal Chemistry. 3, (2002)
2) Gang E, Annan M, Spooner N, Vouros P. Reduction of Signal Suppression Effects in ESI-MS Using a Nanosplitting Device; Anal. Chem. 73(23), 5635-5644 (2001)
3) Arnold D, Needham S. Micro-LC–MS/MS: the future of bioanalysis; Bioanalysis, 5(11) 1329-1331 (2013)

OUTREACH 2013-14

Applied Pharmaceutical Analysis (APA)
Attending
September 16-18, 2013
The Boston Park Plaza Hotel & Towers, Boston, MA

International Society for the Study of Xenobiotics (ISSX)
10th International Meeting
Exhibit
September 28th – October 3rd, 2013
Westin Harbour Castle, Toronto, Ontario, Canada

Chemical and Pharmaceutical Structure Analysis (CPSA)
15th Annual Symposium on Clinical & Pharmaceutical Solutions through Analysis
Shane Needham Program Chair
Short Course: "Method Development for LC/MS: Traditional Approaches and Emerging Trends"
Oral Presentation and Exhibit
October 7-10, 2013
Sheraton Bucks County Hotel, Langhorne, PA

American Association of Pharmaceutical Scientists (AAPS)
2013 Annual Meeting and Exposition
Exhibit
November 10-14, 2013
Henry Gonzales Convention Center, San Antonio, TX

Pittcon
Oral Presentation
March 2-6, 2014
McCormick Place, Chicago, IL

Society of Toxicology
53rd Annual Meeting & ToxExpo
Exhibit
March 23-27, 2014
Phoenix Convention Center, Phoenix, AZ

8th Workshop on Recent Issues in Bioanalysis (WRIB)
Exhibit
March 18-20, 2014
Hilton Los Angeles/Universal City, Universal City, CA

2014 AAPS National Biotechnology Conference
Exhibit
May 19-21, 2014
Sheraton San Diego Hotel and Marina, San Diego, CA

62nd ASMS Conference
Exhibit
June 15 – 19, 2014
Baltimore Convention Center, Baltimore, MD

15th Annual Land O'Lakes Bioanalytical Conference
Attending
July 2014
Fluno Center, Madison, WI

LC/MS DISCUSSION CORNER

Nonclinical Dose Solution Analysis – A Complement to our Bioanalytical Support of Preclinical Programs

Determination of the test article (drug) concentrations in nonclinical dose solutions strengthens the accuracy and thoroughness of in-vitro and in-vivo toxicology studies and is a requirement for GLP studies submitted in an IND dossier. The dose solution analyses have been typically performed at the trial site(s) or at analytical facilities – thus duplicating the technology transfer, validations etc.

Alturas Analytics now offers dose solution analysis services as an extension and complement to the bioanalytical support provided to preclinical programs. Alturas believes that "centralizing" test article (drug) concentration determinations in biological matrices or in dose solutions has several advantages –

- Develops familiarity with the drug(s), assuring continuity of information gained and keeping the science in one place
- Method development, validation and sample analyses performed at one place – irrespective of the matrix (e.g. dose formulation, blood, plasma, serum, urine and/or other biological materials)
- Decreases the method development timeline and technology transfer
- Streamlines and helps resolve analytical difficulties and provides quick solutions
- Helps lower the overall costs for method development

Though the verification of dose solution concentrations falls under the framework of GLP regulations, the only regulatory guidance available is that of bioanalytical method validation. Being familiar with this guidance allows Alturas Analytics to incorporate and address these parameters in the validation of the dose solution analysis method and to perform routine dose solution analysis. The fundamental parameters for bioanalysis and dose solution analysis validations that overlap include: recovery, accuracy, precision, specificity, selectivity, carryover, sensitivity, and stability and are incorporated into the protocols developed for dose solution validations.

With this new service and Alturas' expanding capacity we look forward to providing continuous and uninterrupted GLP bioanalytical support for the drug molecule through its preclinical and clinical development cycle. For more information regarding the dose solution analytical support visit our website at www.alturasanalytics.com.