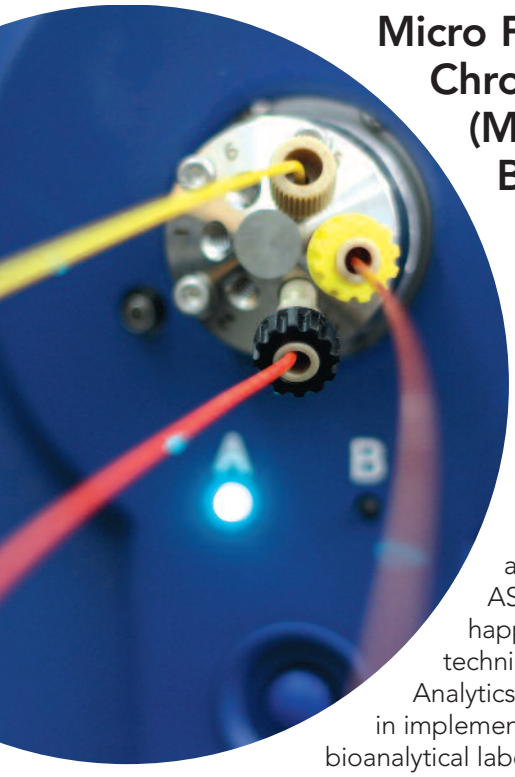
Alturas Advisor

FALL WINTER 2012-13



Micro Flow Liquid Chromatography (MFLC)-MS/MS Bioanalytical Research

Last year in our newsletter, we discussed the enthusiasm we had for our initial research of MFLC-MS/MS use in support of bioanalytical projects. After presenting more data over the past year, MFLC-MS/MS created a big buzz at events such as ASMS and NBC. We are happy to say that interest in the technique is growing and Alturas Analytics continues to lead the way in implementing the technique in bioanalytical laboratories!

Sensitivity of MFLC-MS/MS continues to be a huge advantage and shows typical signal gains of >3-13X compared to traditional flow HPLC-MS/MS (see Figure 1.). In research conducted at Alturas thus far, we see that peptides and large molecules often give better (>10X) signal gains compared to small molecules (3-10X). As our research evolves we are finding that flow rates in the range of 10-40 µL/min are optimal in the bioanalytical laboratory using 0.3 – 0.5 mm ID columns. This scales comparatively with approximate flow rates of 250-750 µL/min on 2.1 mm ID columns.

As MFLC-MS/MS has shown broad implementation in proteomics and academic labs, the biggest question remains, "Can MFLC-MS/MS be used in regulated bioanalytical laboratories?" To determine if the technique can have widespread adoption in a regulated environment, it needs to be demonstrated that the technique is accurate, precise, reproducible and rugged. Additionally, questions regarding potential interruption of workflows arise such as, "Is the source easy to change?", "Does the source require significant amounts of optimization?", "Can conventional HPLC columns and conditions be used?"

To answer the questions listed above, we designed a method validation experiment to compare the conventional HPLC-MS/MS method to a MFLC-MS/MS method for the bioanalysis of methotrexate (MTX) from human plasma. All sample preparation and other method details were identical for each method type; only the HPLC was changed to MFLC. The results are encouraging, with the MFLC-MS/MS method shown to be accurate and precise across all QC levels and with minimal matrix factor (MF) effects (See Table 1.). Additionally, more than 350 samples were injected without a pressure increase; therefore the method was shown to be rugged. Figure 2 shows the overlaid chromatograms from the first and last injections (>350 injections in the run).

Alturas scientists have found several prototype MFLC sources that are easy to change, with the change-over from one to another occurring in less than two minutes. Also, we have found that typical source parameters do not need drastic changes for an optimal method. Previously, the need for marked optimization of source parameters and the large amount of time needed to convert to MFLC were

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STAFF PROFILE:
Becky Koehler

Becky Koehler joined the staff of Alturas Analytics in 2009. As Quality Assurance Manager, her primary focus is ensuring the laboratory compliance with FDA Good Laboratory Practice (GLP) requirements through facility audits, study audits and quality improvement processes. Becky is committed to seeing that all work performed for non-clinical and clinical studies is complete, accurate, and readily understood.

Becky has an extensive quality and regulatory background and has worked in drug development, drug manufacturing and drug prescribing areas of the pharmaceutical industry. She spent 11 years as a QA Manager in two cGMP facilities and 17 years as a Lab Manager and Medical Technologist in three CLIA regulated clinical laboratories before joining Alturas Analytics. She has an Associate of Science degree in Medical Technology and is a dual-agency certified Medical Technologist. She feels fortunate to have completed her degree internship at the world famous Mayo Clinic in Rochester, MN.

Becky lives down the road from Moscow in Lewiston, Idaho with her husband Dave. They enjoy spending time fly-fishing, camping and hiking. She also enjoys reading and hand crafts.

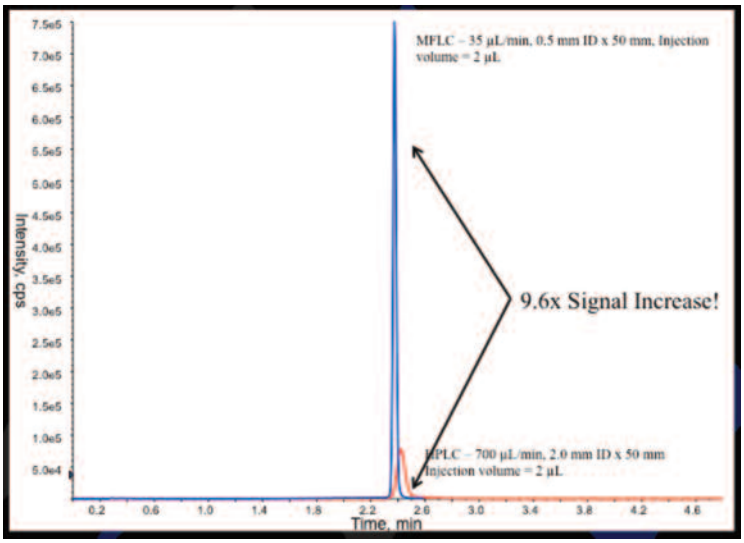

Figure 1. LC-MS/MS Overlayed Chromatograms (HPLC vs. MFLC) from Bioanalysis of Methotrexate (400 ng/mL) from Human Plasma Using Eksigent ekspert microLC 200 and a QTRAP 5500

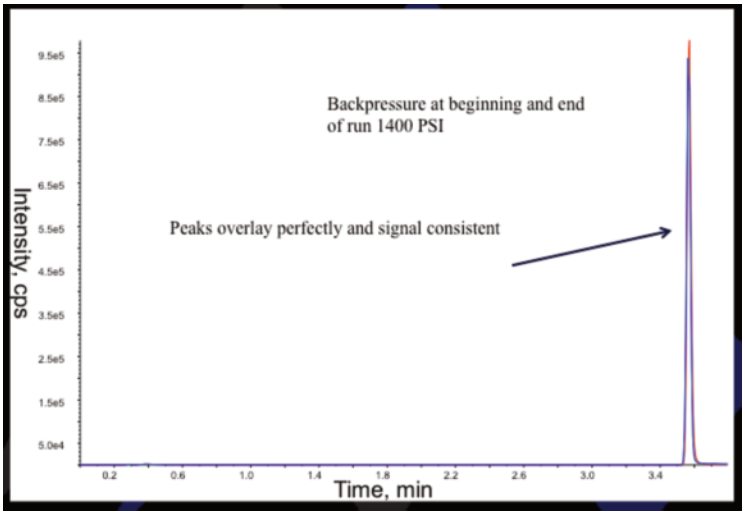

Figure 2. MFLC-MS/MS Chromatograms Overlayed from Beginning and End of Run of >350 injections of MTX Extracted from Human Plasma

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barriers to its widespread adoption in a regulated environment. To further facilitate the use of MFLC, we found that the majority of HPLC column vendors have offerings of sub 0.5 mm ID columns or the willingness to customize a size for you. This is significant as it allows the end user to be able to use the typical column packing materials and stationary phases.

The success of MFLC shown by our team has led to its routine use in our lab. Applications include large molecule bioanalysis, bioanalytical validations and the use of MFLC in dried matrix spot (DMS) analysis. Our scientists recently completed an in vitro study (human liver microsomes) comparing DMS to conventional sampling methods. The experimental design used dex-tromethorophan as a model probe to compare a conventional acetonitrile crash in-vitro sample preparation to a DMS spotting method. The data shows that the methods compare well and DMS could be used as an alternative to conventional in-vitro sampling. In this study, MFLC-MS/MS was used and the MS signal was improved by >4X when compared to conventional HPLC-MS/MS.

As industry advances, the miniaturization and simplification of both instruments and consumables are expected to reduce costs and waste. In line with these expectations, the reduction of solvent use associated with the use of MFLC is attractive. The MFLC technique reduces solvent consumption by >50X when compared to traditional HPLC. In bioanalytical research, we have also discovered that using a switching valve to divert salts and waste during chromatography may not be necessary. This reduces the number of tools needed for bioanalysis and the chance of a switching error by simplifying the instrumentation.

One point of discussion from the bioanalytical community has been that higher column volumes allow higher injection volume for HPLC columns compared to MFLC columns. This often leads to better LLOQs. Although injection volumes are less with MFLC columns as compared to HPLC columns, we have found that relatively larger volumes (compared to overall column volumes) can be injected on the MFLC columns. More research is needed to explain this phenomenon, yet others using MFLC have reported similar results. Overall LLOQs may be similar or better with MFLC compared to HPLC due to this injection focusing effect.

In collaboration with our sponsors and vendors, research continues in our laboratory and includes continued development of sources and chromatographic media to improve the overall signal gains and ease of use of MFLC-MS/MS. For more information regarding our assays visit our website at **www.alturasanalytics.com**.

OUTREACH 2012-13

Chemical and Pharmaceutical Structure Analysis (CPSA)
15th Annual Symposium on Clinical & Pharmaceutical Solutions through Analysis
Short Course: "Method Development for LC/MS: Traditional Approaches and Emerging Trends", Oral Presentation and Exhibit
October 1-4, 2012
Sheraton Bucks County Hotel, Langhorne, PA

American Association of Pharmaceutical Scientists (AAPS)
2012 Annual Meeting and Exposition
Exhibit and Poster Presentation
October 14-18 2012
McCormick Place, Chicago, IL

Pittcon
Short Course: "HPLC Methods Development for LC/MS" and Oral Presentation
March 17-21, 2013
Philadelphia Convention Center, Philadelphia, PA

Society of Toxicology Annual Meeting
Exhibit
March 10-14, 2013
Henry Gonzalez Convention Center, San Antonio, TX

7th Workshop on Recent Issues in Bioanalysis (WRIB)
Exhibit
April 8-11, 2013
Hyatt Regency Hotel, Long Beach, CA

2013 AAPS National Biotechnology Conference
Exhibit
May 20–22, 2013
Sheraton San Diego Hotel and Marina, San Diego, CA

61st ASMS Conference
Exhibit and Poster Presentation
June 9 - 13, 2013
Minneapolis Convention Center, Minneapolis, MN

14th Annual Land O'Lakes Bioanalytical Conference
Attending
July 2013
Devil's Head Resort, Merrimac, WI

Alturas Analytics Adds Protein Binding to Bioanalytical Repertoire

An analyte's affinity to protein binding is an important component in pharmacokinetic and pharmacodynamic activity and is therefore critical for accurate in-vivo modeling. Alturas Analytics now provides protein binding analysis in order to define the nonspecific binding of your analyte(s) to plasma proteins. This service is a great complement to our focus on bioanalytical support of preclinical and clinical programs. Because the protein binding samples are analyzed using the same method as your plasma samples, significant cost savings and greater efficiency are possible.

The protein binding affinity is determined using an equilibrium dialysis device manufactured by HTDialysis LLC. The device facilitates automation in the 96 well- plate format. Following SOPs developed for protein binding, the methods for the innovator molecules are validated along with the plasma or serum method used for the preclinical or clinical study. The innovator molecules are also compared with standard molecules used as positive controls for low, medium and high protein binding affinities.

With this new service and our expanded capacity, Alturas Analytics has the ability and expertise to follow your project from discovery through stage IV clinical trials. To best serve our sponsors, our team will continue to add services in the bioanalytical area. For more information regarding our assays visit our website at www.alturasanalytics.com.

Figure 1: Diagram of a HTDialysis Protein Binding Device