INNOVATOR AWARD ABSTRACTS

Inspiration. Collaboration.

INNOVATOR AWARD SESSION
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Where Technology and Solutions Meet

PicoFuze™: The Future of Microflow LC-MS/MS, "The Column is the Source, the Source is the Column"

[1]Shane Needham, [1]Casey Johnson, [1]Chad Christianson, [2]Stanley Durand, [2]Gary Valaskovic

1) Alturas Analytics, Inc. 2) New Objective

Alturas Analytics has invested significant resources into the development of Microflow Liquid Chromatography (MFLC) for its application in a regulated bioanalytical laboratory. The many benefits of low-flow analytical techniques have been generally acknowledged for quite some time. These benefits include an improved ESI response [1], reduced matrix effects [2] and reduced solvent consumption [3]. As research expands in both vendor and end-user laboratories, the perceived limitations of MFLC, namely ruggedness, robustness, and ease of use, are being addressed and overcome.

Our recent work has continued to demonstrate the benefits of MFLC-MS/MS [3]. Our latest and most exciting research has been an effort of integration, 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. 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 minimized connections will provide optimal MS signal and improve chromatographic peak shape.

For initial experiments, an AB SCIEX 6500 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, source temperature, and ESI voltage, methotrexate extracted from plasma was detected quantitatively. Ruggedness of more than 65 methotrexate sample injections was demonstrated. Again, with minimal method optimization, there was no change in peak shape, signal, or retention time over the sample set, showing promise for the application of PicoFuze™ for high throughput bioanalysis.

Our next step was to compare PicoFuze™ to both conventional HPLC-MS/MS and standalone MFLC-/MS/MS as well. Analysis of enzymatically digested human monoamine oxidase B (MAOB) was used to compare the sensitivity of the three column and ESI introduction formats. 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 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 continues development with PicoFuze™ technology, we are observing considerable signal increases, potentially due to an increase in sampling and/or ionization efficiencies. 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. The many attributes of PicoFuze are such that it's integrated source/column technology is the future of LC-MS/MS for bionalysis.

1) Valaskovix 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. Bioanalysis. *Micro-LC–MS/MS: the future of bioanalysis,* Jun 2013, vol 5 no

Next Generation Plasma Collection Technology For Clinical and Pharmaceutical Applications.

Fred E. Regnier[1], JinHee Kim[1], Tim Woenker[2], Jeff Dahl[2], Jeremy Post[2], Faith Hays[2] and Scott Kuzdzal[2].

[1]Novilytic Laboratories, West Lafayette, IN 47906; [2]Shimadzu Scientific Instruments, Columbia, MD, 21046.

Mass spectrometry (MS) has become a major tool in drug discovery, clinical diagnostics, and personalized medicine. With the rapid progress being made in various modes of mass analysis, MS detection sensitivity, and analyte quantification it is surprising that the accompanying blood collection and sample preparation technologies are so antiquated. Acquisition of blood by venipuncture goes back hundreds of years while the dried blood spot approach of Guthrie is now a half-century-old. The work reported in this presentation will focus on a new technology that exploits the benefits of collection, drying, and transporting small samples on paper; but after the removal of blood cells and collection of a fixed volume of plasma. Subsequent to deposition of a blood drop on a small card composed of a laminated membrane stack (Noviplex cards, Novilytic LLC), the sample rapidly spread laterally by capillary action in the first membrane layer and then proceeds as a front into a second membrane where cells are removed by a combination of adsorption and filtration as plasma is drawn down through the membrane matrix. Migration of plasma through the membrane system terminates in roughly 3 minutes with the collection of either 2.4 or 4.8 uL of plasma in a collection disc at the bottom of the membrane stack. The volume of sample collected between cards varied less that 2% over a hematocrit range from 20% to 71%. At this point the cell bearing upper layers of the membrane assembly were stripped from the card, exposing the plasma filled collection disc to the atmosphere. Within 15 min of air exposure the plasma loaded disc was sufficiently dry to be placed in an envelope for transport by mail or air courier. In both metabolomics and proteomic analysis plasma derived from the plasma extraction card was comparable to that obtained by conventional venipuncture methods.

This presentation will demonstrate the use of this new plasma extraction technology for rapid and reproducible plasma sample preparation and analysis using the latest generation of ultra-fast mass spectrometry. Noviplex cards were used to rapidly generate plasma from mouse whole blood samples for triple quadrupole MS peptide quantitation (Shimadzu LCMS-8050). Exceptional triple quadrupole scan speeds of 30,000 u/sec and the ability to perform polarity switching in 5 msec, combined with rapid and reproducible plasma preparation using noviplex cards, provides a rapid, accurate and reproducible plasma quantitation platform.

Rodent Plasma Micro-sampling Evaluation – Outcomes and Next Steps

Joe Siple – Drummond Scientific

Purpose
To demonstrate the feasibility and comparability of a capillary collection device (75 uL) to a standard collection (200 uL) for plasma isolation in regard to collection and bioanalytical results.

Methods
Two proprietary and one commercially available compounds were dosed to rats in three separate studies. Each study was performed at a different GSK location (UK, PA, and RTP) with different personnel. Blood was collected with both capillary devices and standard collection procedures and subsequent plasma was isolated.

Results
The results of these three comparison studies show, that with the proper training in both sample collection and microvolume sample handling, comparable data can be achieved through the use of the capillary collection procedure. This procedure collects 75 uL or blood, with approximately 40 uL of isolated plasma. Standard assay volumes for this technique were 7 uL.

Conclusion
Utilization of the capillary collection procedure and plasma isolation significantly reduces the volume of blood taken from animal per collection, allowing for the possibility of reduces animal numbers.

NPOI, a novel platform for Biomolecular Interaction Analysis and Affinity Capture Mass Spectrometry.

Keith Waddell, Sergei Malarchuk, Donna Hollinshead
Silicon Kinetics Inc. San Diego, CA

Is it possible to study biomolecular interactions and determine K_D values for binding partners while confirming the molecules that have bound by Mass Spectrometry?

Nanopore optical interferometry (nPOI), is a novel label-free technology for precise measurement of biomolecular interactions. NPOI is based on a unique 3-dimensional matrix prepared by creating a nanoporous region on a silicon wafer. Further processing of the nanoporous structure results in a matrix that is suitable for all classic surface chemistries for binding reactions while maintaining very low non-specific binding. The unique feature of the nanoporous surface is its ability to capture enough material for downstream analysis by Mass Spectrometry. This creates a new approach to Affinity Capture Mass Spectrometry (AC-MS) where the information richness of MS is combined with precise label-free control of the capture process.

The combination of automating Affinity Kinetic measurements with AC-MS now gives a researcher the opportunity to confirm and characterize binding partners and binding properties in a single experiment thus confirming either the validity of an antibody or staging secondary screening for mixtures of compounds or metabolites based on bioactivity.

The NPOI technology used on the SKi Pro® instrument performs fully automated measurements of binding kinetics in multiple samples. The unique SKi Bridge™, a fluidic interface device linking SKi Pro to LC-MS, then narrows the gap between characterizing biological activity and providing structural confirmation.

Typical applications include characterization of protein interactions with other proteins, peptides, nucleic acids, and small molecules.

Perfinity Flash Digest

Kevin Meyer

The Perfinity Flash Digest technology has the potential to simplify applied proteomics much in the same way Taq polymerase simplified PCR. Applications of enzymatic catalyses in biotechnology are often limited by the stability of the enzyme under desired operating conditions. The development of a highly stable immobilized enzyme reactor, combined with an extremely uniform heating apparatus enables the bypass of any sample pretreatment steps and a dramatic reduction in sample preparation times. With the Flash format up to 96 samples are processed simultaneously.

HPLC-HPIMS - A 2D Separation Tool

Ching Wu, Carol Moraff, Anthony Midey

Excellims Corporation

HPLC technology has become one of the most powerful separation technologies; combined with MS, the HPLC-MS is the very successful instrumental analytical method. However, when HPLC is used as a standalone instrument it suffers from relatively low separating power and lacks universal detection techniques. Recent development of electrospray ionization high performance ion mobility spectrometry (HPIMS), has not only made it an alternative detector for HPLC, for UV transparent compounds, but also offers a complimentary separation capability that has resolving power comparable to common HPLC systems. The combination of HPLC-HPIMS will deliver two dimensional separation based on orthogonal separation principles, which can be used as an simple, low cost, compact analytical system in common analytical laboratories.

We have developed an HPIMS system that can accept continuous liquid sample infusion, using an electrospray ionization method to introduce sample mixture to HPIMS. A small part of the HPLC eluents was split and delivered to the HPIMS system, thereby allowing simultaneous measurement of both UV and ion mobility. A two dimensional HPLC retention time and HPIMS drift time plot is used to illustrate the true orthogonal separation capability of the combined system. In addition, attaching the HPIMS to an HPLC-MS system by splitting the liquid flow before the MS, the HPIMS provides a three dimensional separation of sample the mixture.

HPIMS is developed to separate ions formed by electrospray ionization under ambient pressure conditions. The common drift media in this device is filtered air. By eliminating the bulky vacuum components and pumps, the HPIMS could be made into a compact detection system that is based on the same ionization mechanism for LC-MS. Beyond measuring the total ion current generated by ionizing sample components; the ion mobility based separation can be achieved in a time similar to common MS systems, in tens of millisecond; and it is completely compatible with the chromatographic separation retention time. In this study, we used a commercial HPLC system to deliver HPLC separated sample component to the HPIMS for further separation and detection. The preliminary result was obtained using a C18 reverse phase column for amino acid and small drug molecule separation; the co-eluting compounds are further separated based on their ion mobility in air. The two dimensional plot clearly shows that sample components that are challenging to HPLC system can be separated by HPIMS effortlessly.

In common analytical laboratories, separation of isomeric compounds mainly rely on selecting the right column and developing a HPLC method with different solvent combinations and gradients. The method development effort could take up to weeks. Because of ion mobility base separation largely based on molecular size and shape, HPIMS can separate most stereo and constitutional isomers without any method development effort. The study demonstrates at a resolving power of 70 for both HPLC and HPIMS, that the combined HPLC-HPIMS can address the challenges for most isomeric compound separation. Compared to UV detector, the HPIMS based detection system can deliver higher sensitivity; ppb level detection limit was observed.