

HPLC 2014 Conference Review

10 - 15 May 2014, Hilton Riverside, New Orleans, USA

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The 41st symposium of HPLC was held this year in the Hilton Riverside hotel, New Orleans. New Orleans is an ideal location for a large meeting, as it possesses lots of very suitable conference venues as well as a variety of attractions that are highly suitable for a wide range of conference delegates and their partners. These attractions include the river and associated boat trips, casinos, a wealth of restaurants and bars, many that feature musicians and cater for every possible taste, although the choice of exceptionally good steak houses would be too much for most people. The place for souvenirs and other tourists traps is the French quarter which has a lot to offer a worn out delegate.

The meeting itself has seen a slight downturn in attendees in the US version of the show, which is in contrast to the European version which is seeing an increase in numbers. This may be a reflection of the growing use of MS which has meant more US delegates attending ASMS which seems to be getting larger every year. Since there is no comparable size MS event in Europe there is not a draw on the attendees.

The meeting is essential viewing for all chromatographers and is well attended by the leading academics, although it is obvious that industrialists are struggling to open up their wallets. The vendors are still present in reasonable numbers (about 40) but if the downward trend on end users continues it would be expected that their presence will also diminish. The event has very high standards and although there were some logistical issues throughout the week, overall it was well organised and was well received by the delegates. Vendors generally try to launch some products at this meeting, that coupled with the high attendance of leading academics makes it an ideal event for getting up to speed with developments in the chromatography world.

Short courses were given on the Saturday and Sunday, warming up the venue for the opening plenaries on Sunday evening presented by Alan Marshall of Florida State University, Florida on Ultra High Resolution MS for Separations and by Dr. John J. Kasianowitz from NIST, Gaithersburg, Maryland on the separation of Single Molecules using Nanopore Technology. From the Monday through to Thursday, over 200 oral presentations were given, split



between a suite of three rooms with talks in parallel sessions, making this a difficult conference to cover. Over 450 posters were presented in the Grand Ballroom and Grand Salon. To deal with ongoing analytical challenges including foods, pharmaceuticals and the 'omics', many important topics were covered, from separation fundamentals, column technology, novel detectors through to two-dimensional separations. On Tuesday afternoon the Uwe D. Neue Award in Separation Science was awarded to Gerard Rozing a former Agilent veteran chromatographer and now consultant. The Uwe D. Neue Award was created to recognise scientists that have made and continue to make significant contributions to the field of separation science, in honor of the legacy of Dr. Uwe D. Neue, late scientist and Waters® Corporate Fellow.

Daily lunchtime tutorials (some with complimentary New Orleans 'Po boy' sandwiches) included sponsors such as Waters and Supelco, where efficiency was discussed by Fabrice Gritti from the University of Tennessee and an excellent David Letterman-style countdown of 10 HPLC myths was given by Ronald Majors.

Monolithic silica, a competitor of run-of-the-mill columns packed with particles, had dedicated sessions forming a significant part of the whole meeting. It is interesting that this technology has not moved into main stream although it is evident that there are many research groups that are looking to make this happen. One such researcher, Nobuo Tanaka, this year's Chromatographic Society Martin medal winner, of GL Sciences discussed the merits of narrow-bore columns (1 mm internal diameter) either packed with sub 3 micron particles or monolithic silica, emphasising the high demand such techniques place on minimising extra-column volumes to achieve high efficiencies. Kristoff Horman discussed the gain in homogeneity from first to second generation monoliths and limitations of further scaling. Ivo Nischang presented work on polymer, as opposed to silica, monoliths, suggesting that organic solvent acetonitrile is better able to penetrate the mesopores and water is predominantly held in the macropores.

The up-and-coming technique hydrophilic interaction chromatography (HILIC) featured, which retains and separates polar molecules and is a viable alternative to reversed-phase liquid chromatography and enhanced MS performance. David McCalley of the University of the West of England outlined

new developments on HILIC retention and peak shape in his own enigmatic style. Mobile phases buffered with formic acid performed poorly on HILIC columns they studied, with good to excellent peak shapes reported in ammonium formate salt buffer. Selectivities were compared for a type B silica column and type C silica, which is believed to have a stationary phase chemistry of relatively inert silica hydride bonds. Surprisingly, the selectivities correlated well. James Heaton compared performance of HILIC with RPLC, with an in-depth discussion of mass transfer. Comparing results to work published by Gritti and Guiochon and Gert Desmet, surface diffusion in HILIC is perhaps slower than RPLC, possibly due to higher micro-viscosity of the water layer held to the stationary phase. He suggested plate counts for very long columns can be superior in HILIC, and in the 'practical region' of column length, efficiencies are similar between the two techniques but backpressure is much lower in HILIC due to the low viscosity of acetonitrile-rich mobile phase.

There was intense focus the performance of superficially porous particles, which offer high efficiencies and reduced backpressure. Joseph DeStefano of Advanced Materials Technologies discussed the benefits of 2 micron superficially porous particles, suggesting these are optimal over sub-2 micron particles. Kevin Wyndham of Waters suggested packing narrow-bore 2.1mm id columns can be difficult but achievable, with similar efficiencies shown for 2.1, 3.0 and 4.6 mm i.d columns, recommending 1.6 micron particles for UHPLC applications but 2.7 micron particles for established HPLC systems. Wyndham discussed the improved peak shape of charged surface hybrid column chemistry, and improvements in peak shape for ionogenic bases compared to traditionally poor symmetry on C18 columns with dilute formic acid. Browsing the vendor exhibits including Fortis technologies, most column manufacturers now offer modern superficially porous particles. James Grinias of the University of North Carolina presented work to improve the size distribution of silica particles using hydrodynamic chromatography with 32-38 micron glass microspheres, although their throughput is currently somewhat limited to 1.5 mg per hour.

Alternative stationary phases featured, including Chris Pohl of Thermo presenting anion exchange phases by in-step electrostatic graft. Andrea Gargano from the Van't Hoff Institute for Molecular Sciences (University of Amsterdam) discussed the use of mucin-based stationary phases, a useful approach to determine if pharmaceutical aerosol delivery is reduced due to high

affinity for mucus (0.5-5% of which is mucin). This large protein had quite low stationary phase surface coverage, and good correlation between mucin affinity factors and retention for solutes with known affinity factors. James Treadway from the University of North Carolina discussed the synthesis of thin-shell superficially porous particles with wide pores for peptide separations, to deal with the difficulty of large molecules entering standard porous particles. Brett Paull discussed mixed-mode and HILIC phases with a focus on the Trinity series of mixed-mode columns, with charged aerosol detection (CAD); Joachim Weiss of Leopold-Franzens University and Thermo Fisher Scientific also presented work using these columns and CAD.

Ulrich Tallarek described his groups' latest work on modelling the complete chromatographic process, incorporating Giddings' stochastic, probability-based model and limits of bed porosity, sorption kinetics and different rates of interfacial mass transfer. In future, they aim to predict optimum particle types and optimum monolith properties. Eva Tyteca described retention models in HILIC mode, to better meet the expectations of method development, discussing linear, quadratic, and separate models for partition and adsorption separately. Their work suggested predicting elution windows compares favourably to prediction of exact retention times. Georges Guiochon talked about models, myths and mistakes of gradient elution in fast HPLC, describing the build-up of possible 'shocks' from adsorption of sample modifier to the stationary phase. He ended his talk with a somewhat enigmatic warning that empirical models should be interpolated, not extrapolated.

Chiral separations had dedicated lectures. Michael Dong of Genentech discussed the challenges of separating compounds with multiple chiral centres, noting achiral and chiral methods can be useful to separate active pharmaceutical ingredients from impurities and robustness of chiral method development can be a problem (somewhat helped by salt-buffered mobile phases and adding small amounts of hexane). Eva Tesarova of the Charles University in Prague presented on the use of cyclofructan-based stationary phases, describing benefits of adding small amounts of barium (II) to mobile phases to improve separation of compounds containing phosphate groups.

Alternative separation techniques also featured: Govert Somsen discussed the use of capillary electrophoresis coupled to mass spectrometry to determine protein-protein affinity interactions, particularly for pharmaceutical analysis. Microfluidic talks included Detlev Belder from the

Universitaet Leipzig presenting work on chip-based separation devices, using particles or glass chips and detection by MS or fluorescence. Charles Poynik of the Colorado School of Mines discussed Thermal Field-Flow Fractionation, and applied this to separations of high molecular weight polymers as an alternative to gel permeation chromatography. Porous layer open tubular (PLOT) columns, based on the earlier work of Knox and Gilbert, Guiochon, Tsuda and Poppe, were discussed by Barry Karger of the Barnett Institute, Boston. When coupled to MS, he found very low detection limits around the femtomole scale, using very narrow and long tubes (10 micron x 3-10 meters).

Two dimensional separations focused on orthogonality, with talks from Brendan Holland, Michelle Carmenzulli and Fahimeh Kamarei. Michelle discussed a new model for determination of orthogonality, and it is clear that there is still some work to be done in this area regarding maximising information gathered in 2 dimensional separations. Peter Schoenmakers ran through his groups' progress on 2D separations, aiming for optimal peak capacity in the minimum analysis time and emphasising the need for compatible solvents between the first and second dimension (HILIC and RPLC – good; NPLC and RPLC – bad).

Possible approaches to counter frictional heating, blamed for possible loss of efficiency and unexpected changes in retention, were covered by Jason Anspach from Phenomenex and Frank Steiner from Thermo. Steiner suggested still air column heating performed better than forced air circulation, and Anspach suggested loss of retention at high flow rate could be more pronounced for short than longer columns. It was interesting to note that Frank's presentation had several slides of what appeared to be a new instrument, which was duly launched shortly after the show (the Thermo Fisher Scientific Vanquish UHPLC).



Applications which were more diverse included talks by Paul Haddad of the University of Tasmania and Deidre Cabooter of KU Leuven. Haddad presented work on analysis of anions and cations applied to explosives, for pre and post-blast analysis in the field. His group had found ion chromatography (IC) and CE to be complementary techniques, with IC sensitive and robust for cations, whereas CE was faster with better peak capacity. Cabooter presented a proof of principle use of HILIC and RPLC columns with novel flow switching valves, applied to analysis of pharmaceuticals in waste and surface water; when optimised, they achieved a separation of polar and apolar solutes within 30 minutes. Robert Kennedy of the University of Michigan presented work on their droplet CE, including a 'sipper chip' for high-throughput screening in 96-well plate formats. Kennedy presented work on a chip-based Western blot, potentially a huge improvement for biologists using the ubiquitous but slow traditional technique. Guibin Jiang discussed some approaches to separation and identification of persistent organic pollutants, including

perfluorooctanesulfonic acid (PFO's) and short chained chlorinated paraffins (SCCP's). Norm Dovichi of the University of Notre Dame and Gerard Hopfgartner of the University of Geneva discussed 'omics' studies, where traditional chromatography has limited power to help achieve goals of finding the most proteins and high resolution MS is crucial. Mary Wirth also presented an update on the slip flow technology, which allows for the use of sub 1 μm particles without excessive pressures, allowing columns up to a few meters in length to be used with incredible efficiencies being obtained. Organised social gatherings included a welcome reception in the Hilton on the Sunday and the vendors reception on the Tuesday. Impromptu events included micelle-like groups passing through the French quarter, taking in the sights (and stench) of raucous Bourbon Street before settling in venues offering New Orleans raspberry beer, slush machines filled with premixed daiquiri, local cuisine and renditions of Dr. Johns' 'Right place, wrong time'.

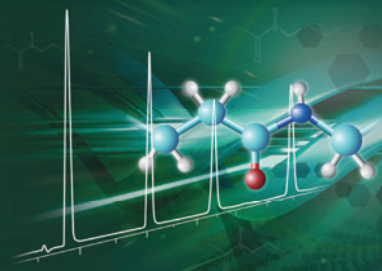
The conference dinner was well-attended, and took place on the 'Natchez' steam boat.

A jazz band provided background music whilst the boat did laps up and down the downtown area on the Mississippi. The wine left a little to be desired, but the selection of roast beef and bowls of gumbo provided some value. Some danced, some walked the decks taking snaps of the evening lights with their iphones then most headed to the bars of Frenchmen Street.

The Agilent best poster awards were presented on in the closing ceremony, with Stephen Groskreutz of the University of Pittsburgh winning first prize for modelling temperature assisted solute focusing in capillary HPLC. The attendees were invited to future HPLC conferences in Geneva 2015, Beijing late 2015 and San Fransisco in 2016. I'd like to give personal thanks to Greg Jonas of the Chromatographic Society for arranging a generous bursary and the John Dolphin Fellowship. I strongly recommend young separation scientists to apply for this and get the opportunity to attend such prestigious conferences and meet the researchers in person whose names come up on papers and webcasts.

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