ASMS LC-MS & Related Topics Workshop and Interest Group Meeting Summary, 2012

Presiding: Susan E. Abbatiello, Ph.D. (The Broad Institute of MIT and Harvard)
Date: Tuesday May 22, 2012 (5:45 pm)
Attendance: Approximately 300 (standing room only in conference room 220-222)

Workshop Summary:
The agenda for the 2012 Workshop for the LC-MS & Related Topics Interest Group at ASMS in Vancouver was as follows:

5:45-5:50 pm: Introduction and Announcements
5:50-6:15 pm: Graduate Student Presentations
6:15-7:00 pm: Open discussion on LC-MS Troubleshooting topics, moderated by 4 panelists

Student Speakers:
Jordan Stobaugh, University of North Carolina, Chapel Hill
Joshua Buse, University of Saskatchewan

Panelists:
Thomas Blau (Ion Technology Support, Inc)
Helene Cardasis, Ph.D. (Merck)
Brent Dixon, Ph.D. (Physicians Choice Laboratory Services)
J. Will Thompson, Ph.D. (Duke University)

Student Presentations:
Based on a poll conducted through the LC-MS Interest Group in February 2012, 70% of the responding participants indicated they would like to see two graduate student presentations at the beginning of the workshop. In order to focus the presentations on LC-MS troubleshooting topics, we asked the graduate students to adhere to a PowerPoint template (Appendix 1). Graduate students were selected by filtering the accepted poster abstracts by "graduate student presenter", then further filtered to ensure the poster topic was related to LC-MS research. Eighteen graduate students were contacted individually and told they were on a list of 18 finalists for 2 open presentation slots at the LC-MS & Related Topics Interest Group Workshop. Eleven students responded that they were willing to participate and their abstracts were ranked by the four panelists participating in the workshop. The two students with the lowest combined score (indicating best ranking) from the ranking step were Jordan Stobaugh and Joshua Buse. Each student delivered an oral presentation of approximately 8 minutes, allowing for 2 minutes of questions.

Jordan Stobaugh, University of North Carolina, Chapel Hill, "Protein Identification vs Run-Time Comparison between Nanoscale 2D-LC-MS and a 1D Two Meter-Long Microcapillary Column for Complex Samples"

Jordan weighed the pros and cons of peptide separations by LC-MS using either a 2D separation with 5 fractions and 30 minute gradients or a 2 meter long 1D separation for 5.5 hours. The 1D separation was conducted at high pressure (30,000 psi and greater) and maintained at constant pressure. His results demonstrated using a 1D separation allowed for maximization of the column peak capacity from ~140 for each 2D fraction separation to ~1000 for the 1D separation on the long column. Limitations included operating at very high pressures, and while operating at constant pressures, leak detection can be difficult.

Joshua Buse, University of Saskatchewan, "Comparative analysis of four mass spectrometric methods for the quantification of drug delivery agents: bisquaternary ammonium gemini surfactants"

Joshua's presentation focused on evaluation of LC-MS, FIA (flow injection analysis)-MS and MALDI-ToF MS and comparison of the pros and cons of each method for quantification of the target surfactants. The FIA-MS method achieved the lowest limit of quantification, MALDI-ToF MS achieved the largest linear range of detection. The LC-MS method not only had a smaller linear range (2 orders of magnitude), but higher costs associated with solvent consumption and analysis time. The conclusions of the work were to use MALDI-ToF MS for quantification of the surfactants.

Workshop Topic: "Hot Topics in LC-MS Troubleshooting"

The topic of this year's workshop, the same running topic for the last 2 years, was meant to help the attendees identify and address any problematic areas in either chromatography or mass spectrometry, and to bring these topic s up for discussion in the audience and with the guest panelists. A slide with several "troubleshooting topics" and associated diagnostics was presented to help define problem areas in LC-MS and to encourage discussion (Appendix 2).

The discussion began with some anecdotal experiences with LC-MS instrumentation difficulties from Tom Blau, owner of Ion Technologies. Tom shared experiences of troubleshooting MS instrument performance to narrow down the cause of certain problems, which may be common in various research labs.

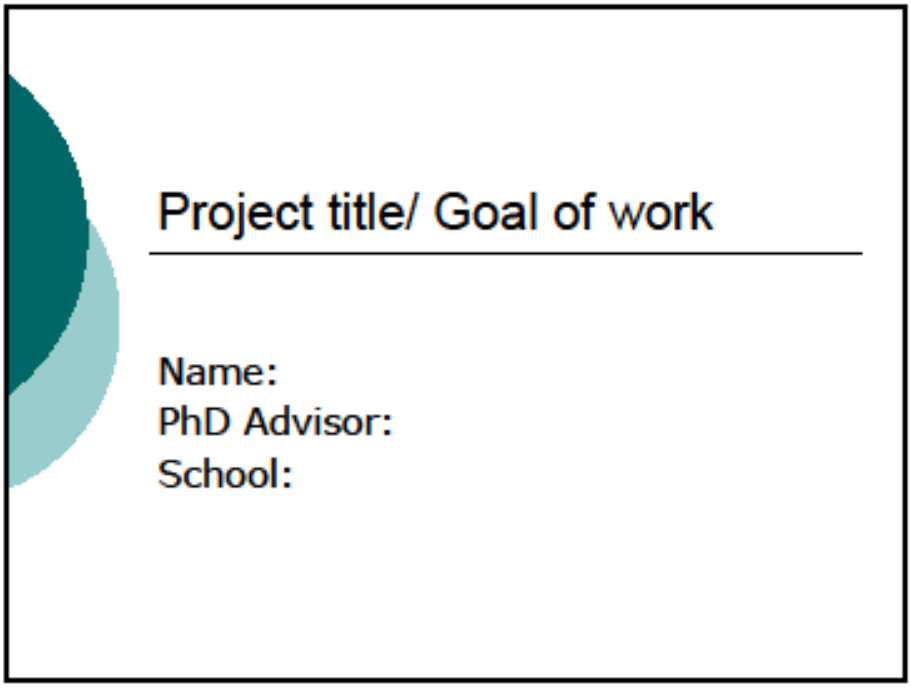
In order to get discussion rolling, we asked the audience to think about what maintenance or repair steps do they normally call a service engineer in to perform that they would like to learn how to do themselves. General feedback centered around cleaning the ion source of the MS, changing check valves on HPLCs, and having a general workflow for troubleshooting low or no signal in the MS from a sample introduced by LC.

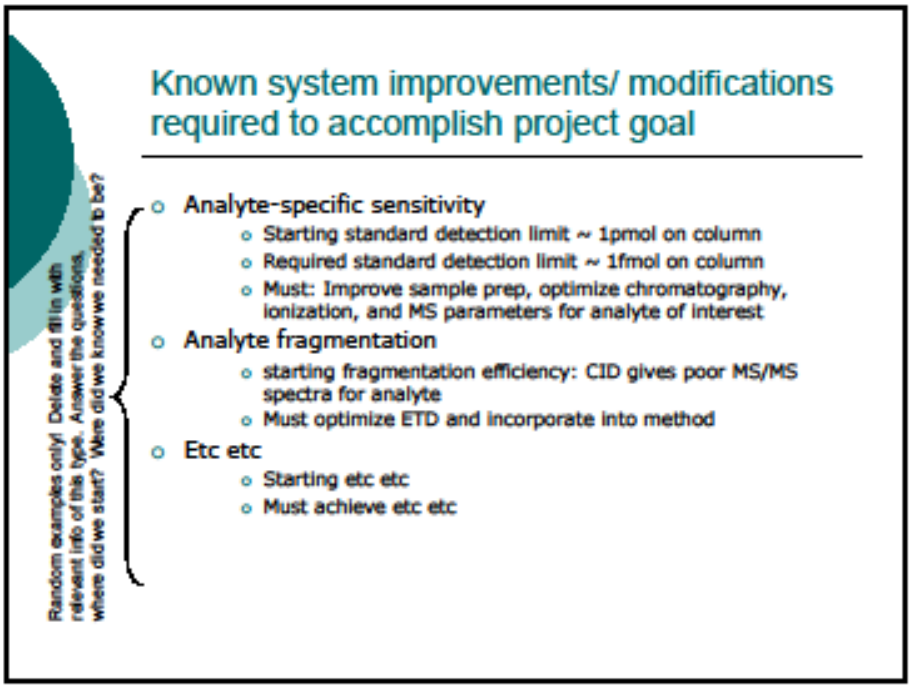
General audience questions seemed to concentrate on LC-related topics: the use of trapping columns, column longevity and performance, how to deal with sample carryover, etc. MS related questions came up when an audience member described very weak signal for a standard sample from an LC-MS experiment. Suggestions from the panelists and the audience included decoupling the LC from the MS and infusing the standard directly into the MS to make sure the MS performance is within expected specifications.

As with the workshop in 2011, questions increased in their frequency toward the end of the workshop. However, this year's workshop had a much smaller lag in audience participation than in 2011, and audience members seemed generally willing to ask questions and offer their own advice and experiences. At the beginning of the workshop, the room was filled to capacity with many 10's of attendees standing. At the end of the workshop, few people had left and the room still appeared to be full to capacity.

An additional comment from attendees was in relation to an on-line forum through which trouble shooting discussions could take place among ASMS members and practitioners of LC-MS. While ABRF has a very similar forum, based on the interest from the workshop attendees, it may be something worthwhile to initiate on the ASMS website.

Appendix 1: PowerPoint Template for Graduate Student Presenters





Issues addressed along the way

Random examples only! Delete and fill in with relevant info of this type. Be candid and forthcoming (we've all been there!)

- Sample not stable in autosampler over 4 hours
- Retention time shifts and band broadening over course of sample queue
- Sample matrix caused column clogging
- Strange software error messages overusing word "fatal"
- Default MS tune parameters far from optimal, actually debilitating
- Matrix interference
- Ran out of sample mid-way through experiment, second sample set not consistent with first
- Hydrophobic analytes retained on autosampler needle, resulting in low signal in sample runs, high carry over in wash runs
- Low concentration sample sticks to autosampler vials
- Standard samples worked fine, study samples did not.
- Forgot to clean my heated transfer capillary for 2 months

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Solutions

- Sample prep:
- LC:
- MS:
- Software:

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Appendix 2. Discussion Topics for LC-MS Troubleshooting

Troubleshooting Topics

Problems

- Chromatography
  - Autosampler injection reproducibility
  - Temperature control
  - Column aging, clogging
  - Fluidic connections
  - Peak widths
- **ESI**
  - Electrospray stability
  - Loss of sensitivity
  - Dirty source
- **MS**
  - Tuning
  - Calibrating
  - Sensitivity, signal
  - Chemical, shot, environmental noise

Diagnostics

- **Chromatography**
  - Pressure trace
  - Temperature monitoring
  - Data analysis software
  - System suitability
- **ESI**
  - System suitability
  - Data analysis software
- **MS**
  - Vendor tools
  - System suitability
  - Data analysis software