ASMS 2013
LC-MS Troubleshooting Workshop

Chair/ Co-chair:

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Expert Panel:

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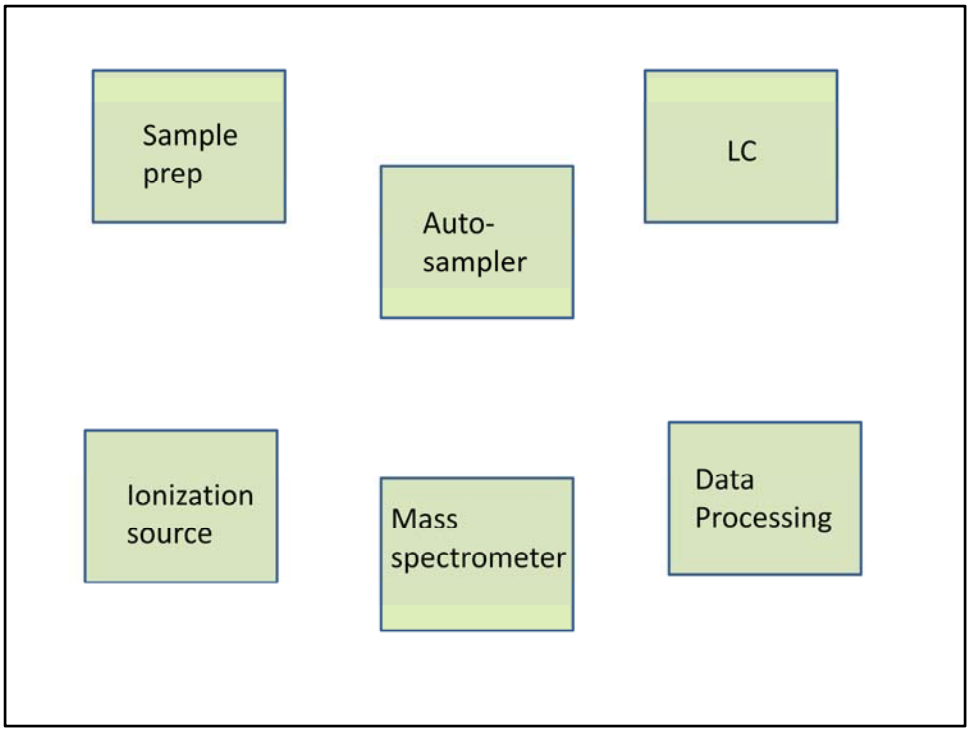
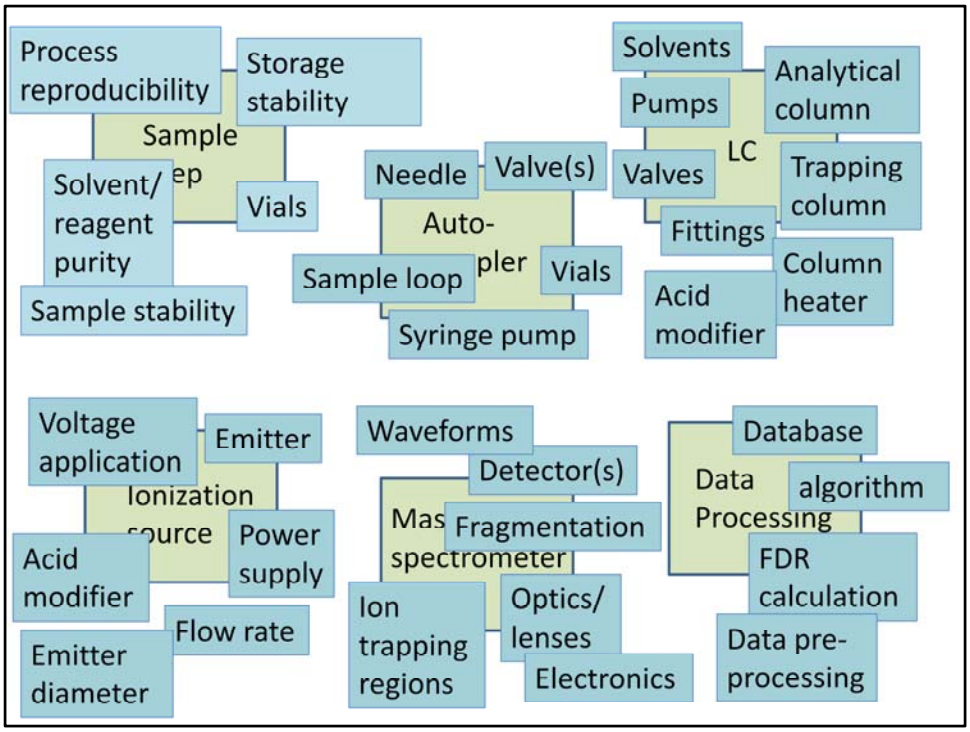
Brent Dixon, Chief Scientist, Physicians Choice Laboratory

Michael Bereman, Staff Scientist, University of Washington


Sample prep
Auto-sampler
LC
Ionization source
Mass spectrometer
Data Processing


Process reproducibility
Storage stability
Sample
ep
Solvent/ reagent purity
Vials
Sample stability
Needle
Valve(s)
Auto-
pler
Sample loop
Vials
Syringe pump
Solvents
Analytical column
Pumps
LC
Valves
Trapping column
Fittings
Column heater
Acid modifier
Voltage application
Emitter
Ionization
source
Power supply
Acid modifier
Flow rate
Emitter diameter
Waveforms
Detector(s)
Mas
spectrometer
Fragmentation
Ion trapping regions
Optics/ lenses
Electronics
Database
Data Processing
algorithm
FDR calculation
Data pre-processing

Deconstruct the problem

- **Primary symptom** – points directly to potential source(s) of problem
- **Secondary symptom** – vague… requires further investigation to identify underlying primary symptoms and determine source.

high variability in peak area of a standard sample injection to injection

Primary – likely sample delivery issue. check pressure trace, autosampler needle, vial depth, syringe, valves, sample loop, loading/trapping column, spray stability…

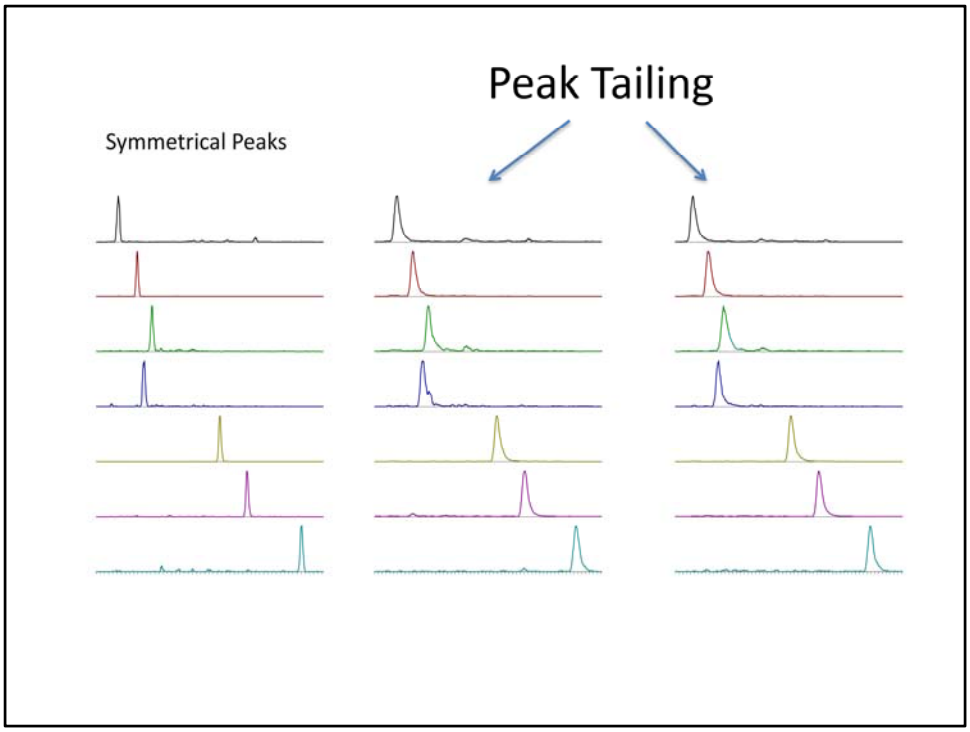
Exception: Does trend in one direction? if change/trend in one direction…maybe mass spec

No signal

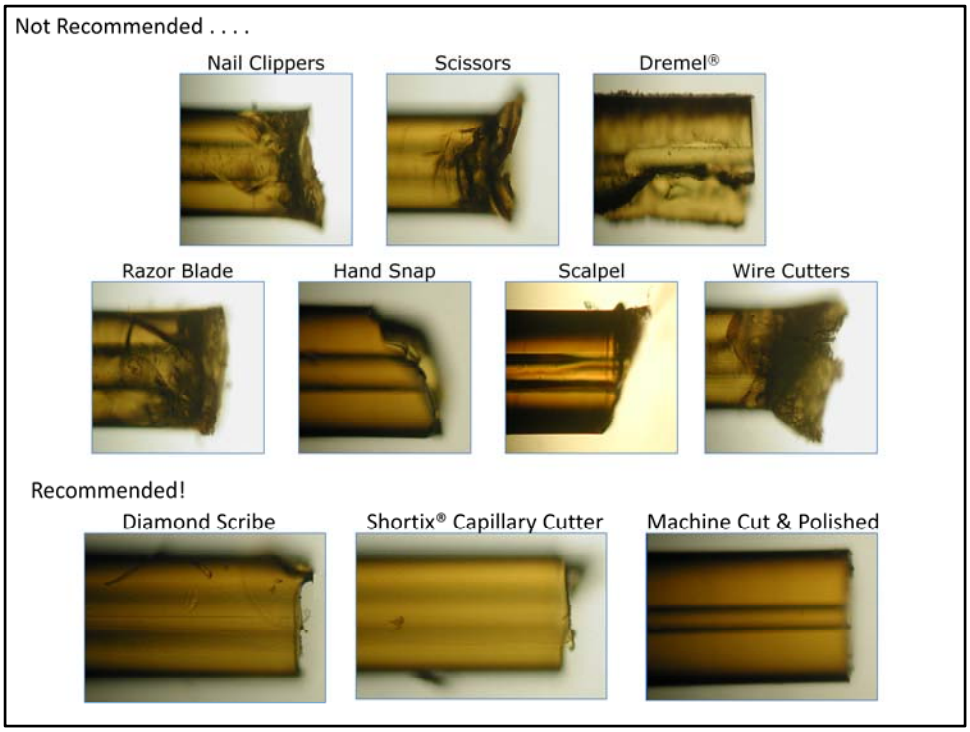
Secondary – no sample signal? No chemical signal? Only electronic noise? Check 1) sample, 2) sample delivery, 3) ionization, 4) MS direct infusion

Reduced peptide spectral matches (PSM)

Secondary symptom – could be anything. Check 1) sample, 2) sample delivery (AS injection, LC trapping, LC/ column performance (elution range, peak widths, tailing?, pressure traces)), 3) check ionization/ spray stability, 4) check MS direct infusion



Primary symptom – bad connections made after trap or analytical column (leak). Check these connections. Degraded sample, buildup on head of column (would see in pressure trace)

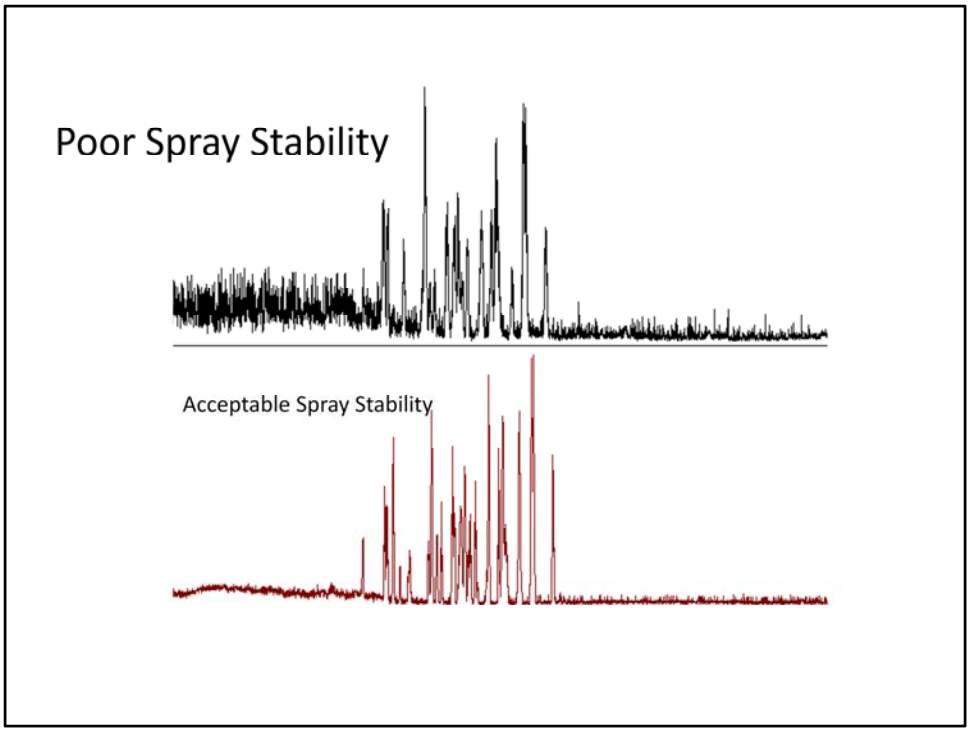

Not Recommended . . . .
Nail Clippers
Scissors
Dremel®
Razor Blade
Hand Snap
Scalpel
Wire Cutters
Recommended!
Diamond Scribe
Shortix® Capillary Cutter
Machine Cut & Polished

Reduced signal in MSMS,
no loss of signal in MS1

Primary, but can go one step further and check isolation efficiency to determine if the issue is isolation or actual fragmentation. If multiple fragmentation modes and detectors exist, be mindful of whether loss of signal in MSMS is in only one or all MSMS modes, and in only one or all detectors.

“My unknown looks bad.”

Secondary – come on. Run a standard.



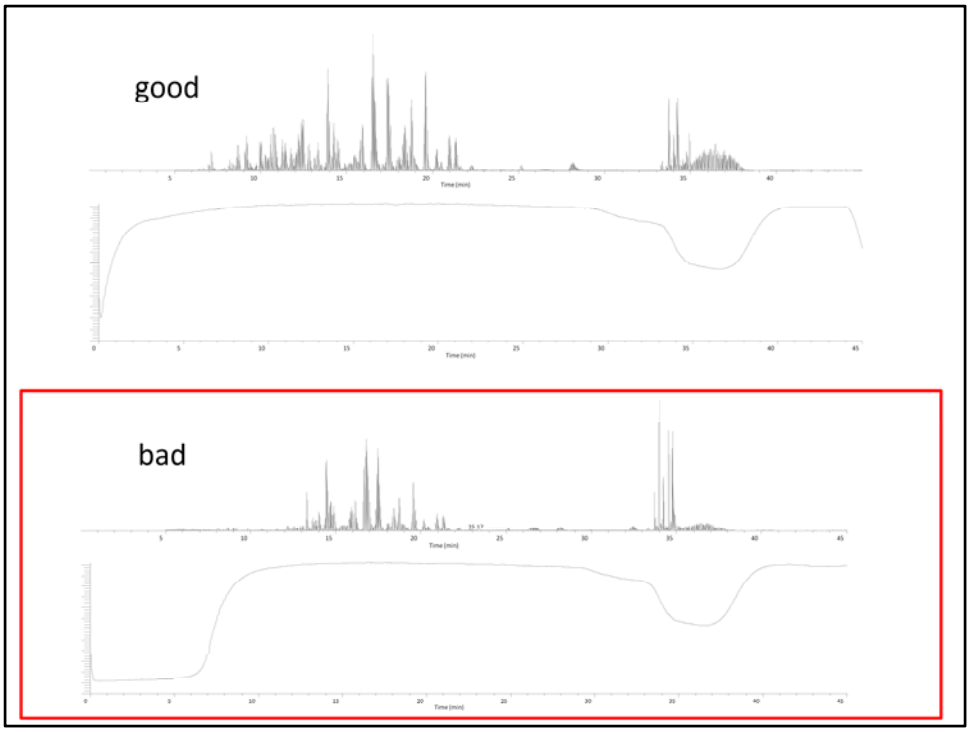
Primary – is it spray related, flow related or MS related? For nanospray – visualizing the spray is key to troubleshooting. Troubleshooting – increase or decrease the voltage – do you see a response? Actuate the valve – does the signal drop to zero, improve or get worse – indicating a leak in the valve flow path, pump isocratically to distinguish changes related to mobile phase composition. Check site of voltage application (flush liquid junctions, ensure proper contact for contact based junctions), check connections, check emitter tip wear (change tip).

NO filtered 18 Mohm, deionized water WATER!!!!!!!!!!!!!! USE HPLC GRADE BOTTLED WATER!!!!!!!!!!!!!!!!!!

Long dips in LC pressure profile after valve actuation

Primary – leak in valve, check connections to valves, make sure capillary end is flush with sleeve and sleeve/PEEK is properly spaced from end of ferrule, tighten connections, change/clean rotor, clean stator

If no pressure profile… how does this manifest in MS data?


good
bad

Even reduction in sensitivity across all scan events

Secondary symptom – check 1) fresh sample, 2) check sample delivery (AS diagnostics, bypass AS and bomb load, different injection volumes), LC/column (change column, trap, run LC diagnostics, swap LC if possible), 3) ionization (check spray stability), 4) run MS diagnostics

Check MS by nanoflow infusion to keep source consistent… very helpful to find a peptide mix that can be both infused and injected via autosampler/LC system.

Retention times shifting earlier

Primary – could be column not equilibrating properly, could be problem in mixing tee, leak, clog, overloading, mobile phase delivery, different sample composition…not binding, old column

“Decreased sensitivity”

Secondary symptom, same as reduced PSMs. Check 1) sample, 2) sample delivery (AS/injection, LC/column), 3) ionization, 4) MS

Retention times shifting later

Primary – clog or leak in B line, leak anywhere, air in line/ valve, column aging (see pressure trace)

MSMS spectra contain only electronic noise

Primary – does MS1 look ok? Does isolation only look ok? Bad in all detectors? Bad in all modes?

How do you determine whether you're looking at electronic and chemical noise? Electronic noise won't give fragmentation spectra (isolate and fragment), electronic could be a harmonic… isolate an actual peak… does it still show up and at some mathematical distance from actual peak?

Noisy Data

Secondary – result of unstable spray, dirty optics in MS or contaminated LC mobile phase or lines. Troubleshooting – change mobile phases, purge/clean lines and sample loop, infusion to isolate LC and/or AS from flow path; clean MS optics; is the noise consistent or specific to intense analytes or specific scan modes. Validate spray stability visually; if visualization is not an option, increase the spray voltage to see if response improves.

High background in base peak chromatogram

Secondary - Same as noisy data, isolate problem to sample, LC, source, or MS

Low S/N for high charge ions relative to low charge ions

Secondary – could be sample delivery: are high charge species as abundant as low charge species in sample tested? Test that S/N looks ok by direct infusion for a tune mix with equal concentrations of low and high charge peptides. If ok by infusion, check same sample infused on column… column losses? Binding to AS needle? If not ok by infusion, tune on high charge ions for improved transmission, try various scan speeds/ scan types. perform all related MS calibrations, report continued issues to vendor FSE.

Reduced sequence coverage of BSA

Secondary, same as PSMs and sensitivity drop. Check 1) sample, 2) sample delivery (AS/injection, LC/column), 3) ionization, 4) MS

Cleaning resolves performance issues only for a short time (days-weeks)

Primary – spray issue? If yes, fix spray. If not MS charging… reverse polarity testing…

Mass accuracy drifts quickly

Primary… check water chiller/ room temp/ detector issue/ electronics

Signal in positive mode but not negative mode

Spray, analyte/ correct modifier/ lc system, calibration in neg current? All power supply diagnostics pass?

Especially important w polarity switching… choose right modifier

Poorer resolution in ion trap spectra

Primary – check Helium/ Argon/ damping gas

Poor resolution in Orbitrap spectra

Primary – ctrap n2 damping gas, not enough (not trapping) or too much (killing transient)

Poor resolution in TOF spectra

Electronics (pulsing), vacuum (gas)

Also mass shifts!

Poor intact protein fragmentation spectra

Secondary – Good intact detection? Optimize pressures/ voltages for intact transmission and trapping/isolation, then optimize fragmentation for bigger stuff.

Resources

- http://www.asms.org/member-center/interest-groups/lcms-and-related-topics (where these slides and list will be posted)

- http://www.lcresources.com/resources/TSWiz/TroubleShootingWiz.htm
- http://www.chromacademy.com/hplc-training.html
- http://www.chromacademy.com/mass-spec-training.html
- http://www.newobjective.com/electrospray/index.shtml
- http://www.abrf.org/index.cfm/list.index
- http://peptide.nist.gov/software/nist_msqc_pipeline/NIST_MSQC_Pipeline.html