Practical LC-MS Troubleshooting and Maintenance: A Primer

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General Troubleshooting Guidelines

Visual Inspection is Key!

Start By “Breaking the System Down”

Having a BASELINE for all metrics is critical to proper troubleshooting

- -Logbooks to track problems and solutions
- -Reference Values for Instrument Readbacks
- -System Suitability

“Break the System Down”

Review All Available Data
Chromatogram, Mass Spectra, Pressure Traces, Error Logs

Isolate Major System Components
Mass Spectrometer (Infusion Analysis)
LC (Standard Mixture)

LC System
Critical Tool:
Standard Mixture Separation!
Critical Readbacks:
Pressure Traces
Flow measurement
Injection Check

Isolate Problem To:

Column(s)	Tubing
Autosampler	Injector
Valves	Pump

Mass Spectrometer
Critical Tool :
Infusion!
Critical Readbacks:
Voltages
Vacuum Gauges
Detector Signal

Isolate Problem To:

Ionization/Source	Vacuum
Calibration	Mass Analyzer
Detector	

What to Learn From Your Engineer

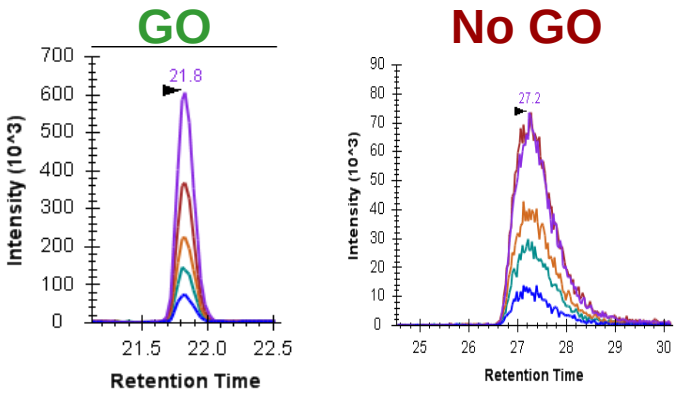
The time to gather key information to enable troubleshooting is at the time your instrument is installed… "**C**ompare to **I**nstallation **V**alues" (**CIV**)

- Vacuum Settings (all regions)
- Voltage Readbacks (screenshots of "good" values)
  - Source
  - Boards
  - Power Supplies
- Tune Mixes to Use (and "good" values)
  - Sensitivity, Resolution, Stability
  - Mass Calibration, S/N
- AutoTune/Calibration Best Practices
- Chromatographic Performance
  - Pressure Range for X Column
  - Peak Width/Resolution of critical pr
  - RT Stability
- Error Log Message Descriptions
- PM Schedule for Your "Use-Case"
- How Best to Find Spare Parts
- Restrictions on Solvents, pH, etc with your LC

Also, your engineer probably has troubleshooting documents that are "not widely distributed". Ask for them! (It can't hurt)

What is System Suitability?

- "System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated". (FDA)
- "The checking of a system, before or during analysis of unknowns, to ensure system performance" (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceutical for Human Use [ICH])
- **Simply put: Analysis of a known sample to assess system performance**
  - Helps to identify when the system is not working



Define Pass/Fail Criteria

What are metrics of a system suitability protocol?

MS

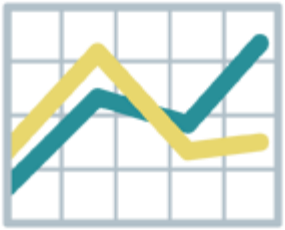
- MS response, sensitivity
- Mass accuracy
- Precision

LC

- Retention time
- Peak shape, FWHM
- Chromatographic resolution

How can you assess “normal” system performance?

- Tune/calibrate LC and MS components
- Monitor variability of metrics over several injections
- Always use the same method for LC and MS, even if it is different than your sample method
- Ask the vendor for performance specifications for both LC and MS parameters
- Note when system performance deviates
- Compare to other systems running the same system suitability protocol



How can you evaluate system suitability data (for free)?

- RawMeat.exe*: vastscientific.com/rawmeat/
- NIST metrics: peptide.nist.gov/metrics/
- Skyline: proteome.gs.washington.edu/software/skyline
- Panorama: www.panoramaweb.org
- Retention Time Viewer: gibsonproteomics.org/resources/rt-viewer
- Database searches for peptide ID
- Excel
- Any longitudinal data tracking system
- Check with your instrument vendor

* Thermo instruments only

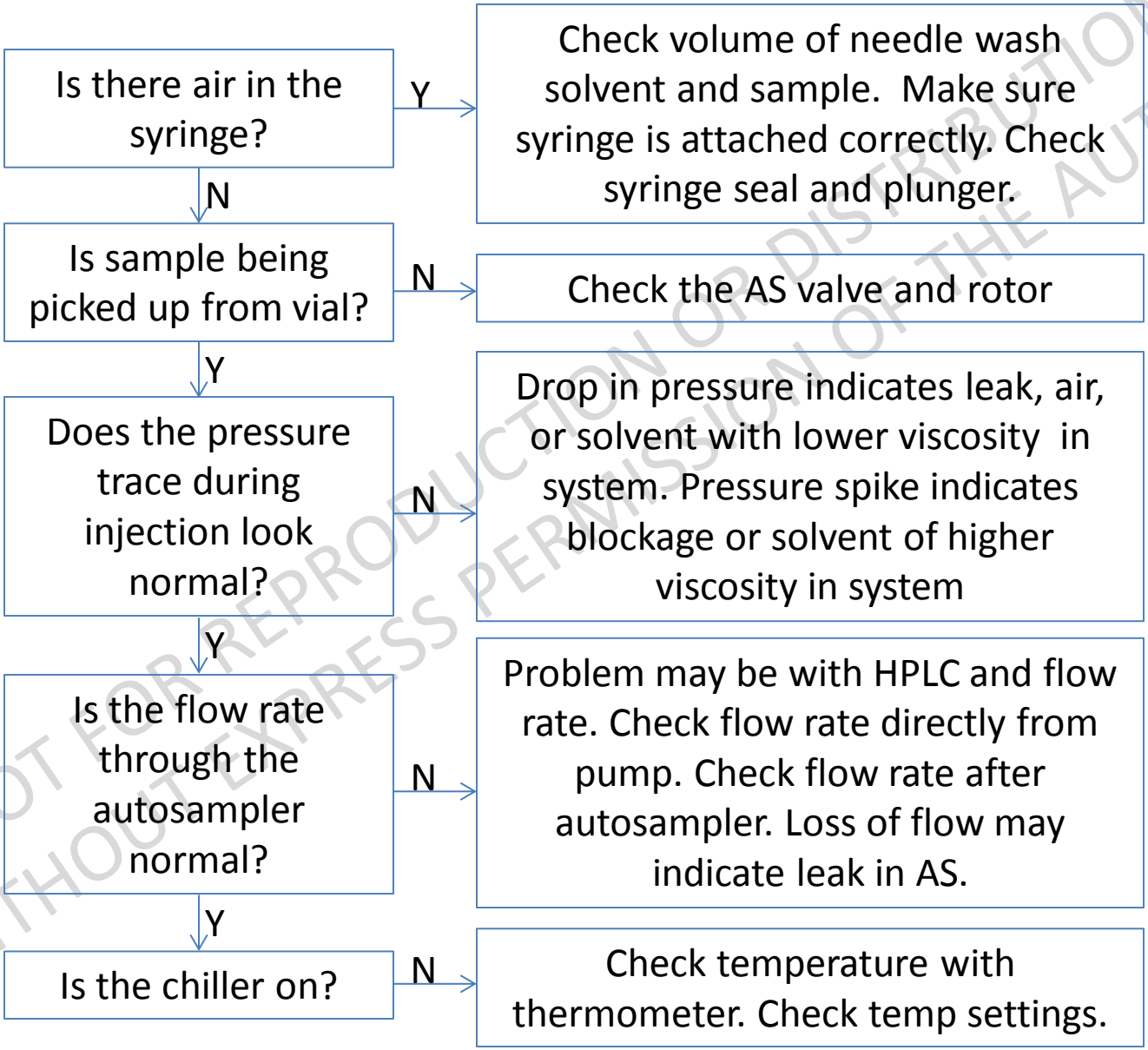
Summary

- Set up a system suitability protocol that works for you and use it
- Don’t waste precious sample without making sure your LC-MS system is working
- Use system suitability to monitor changes in hardware, software, any changes at all
- Keep examples of poor system suitability data to help trouble shoot future issues

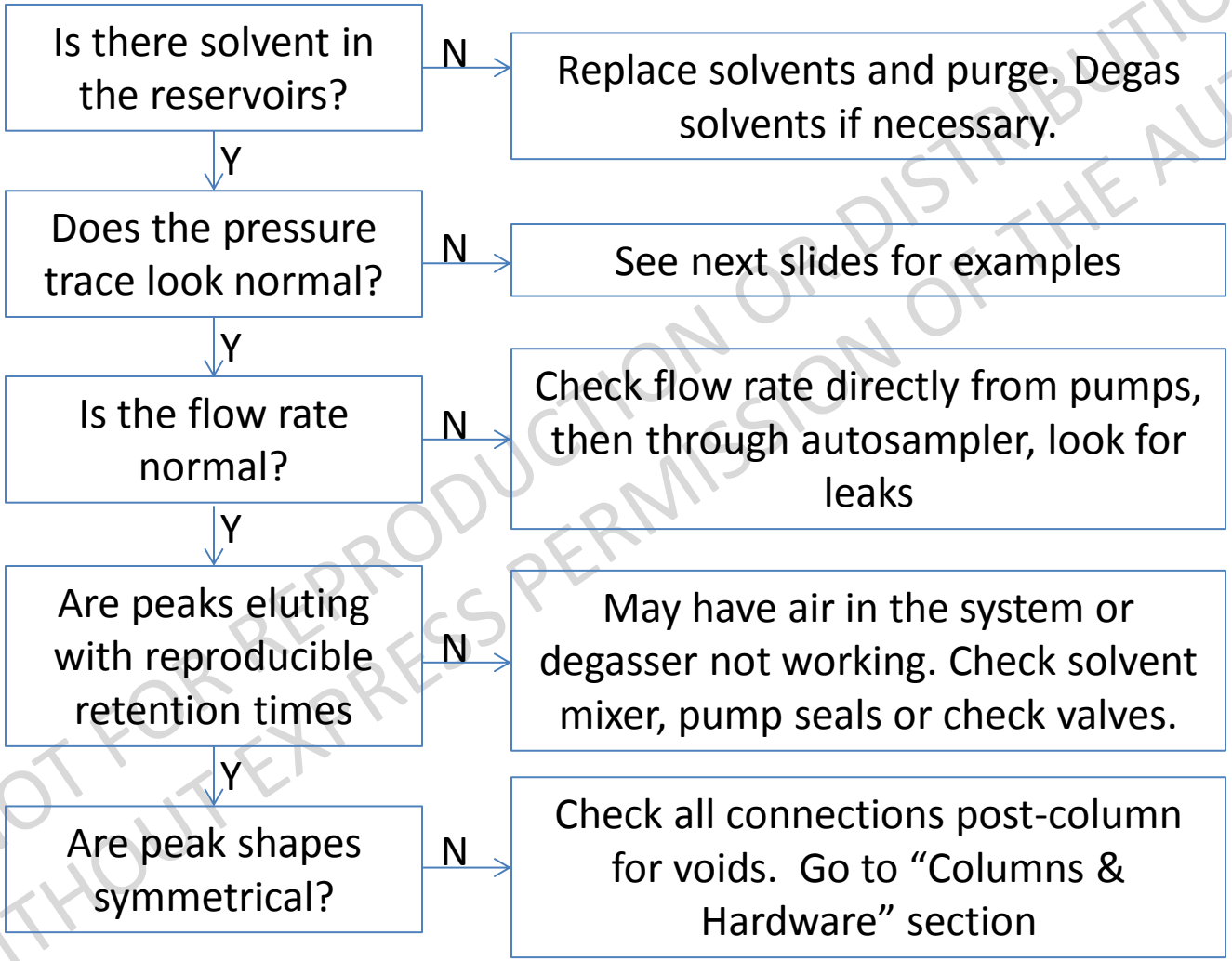
General LC Troubleshooting

Tips, Tricks & Maintenance

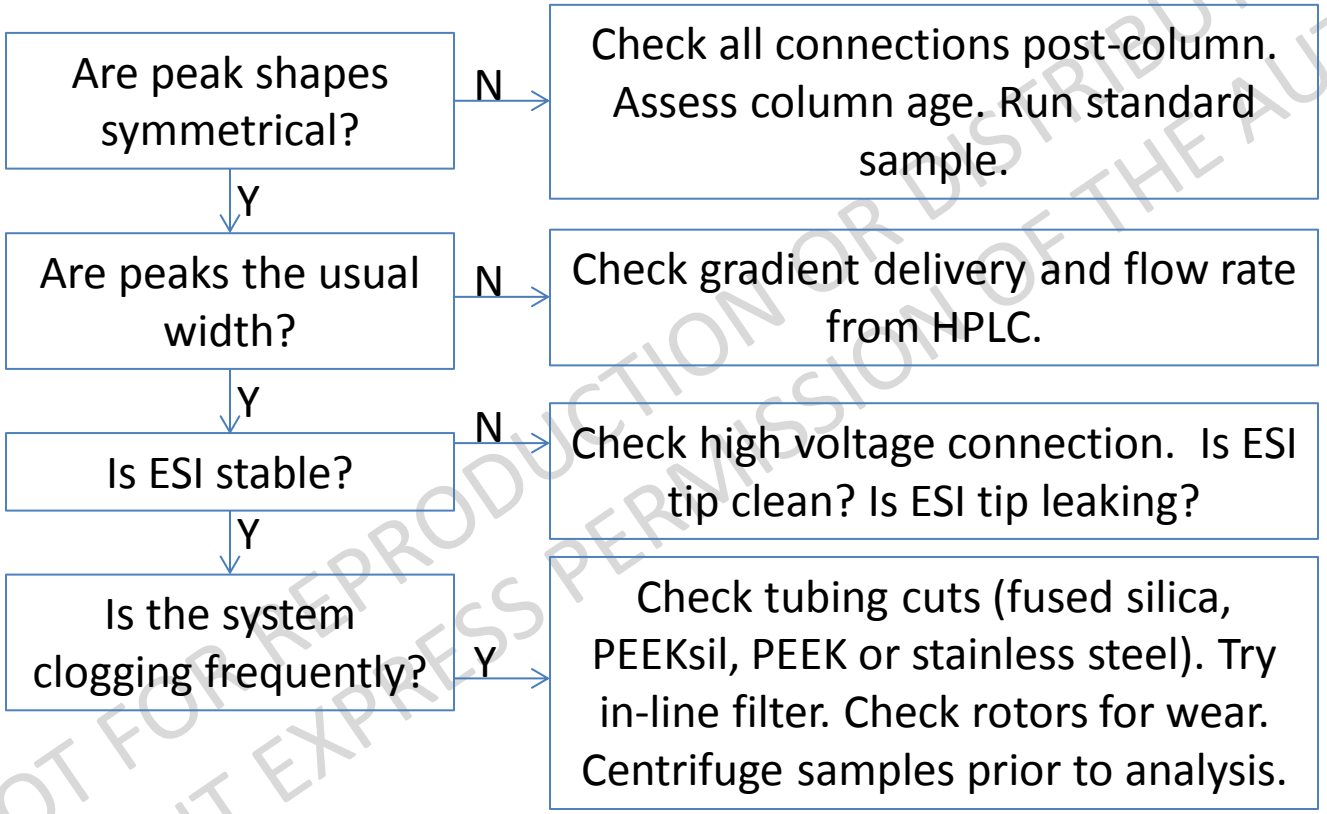
Autosampler



HPLC Pumps

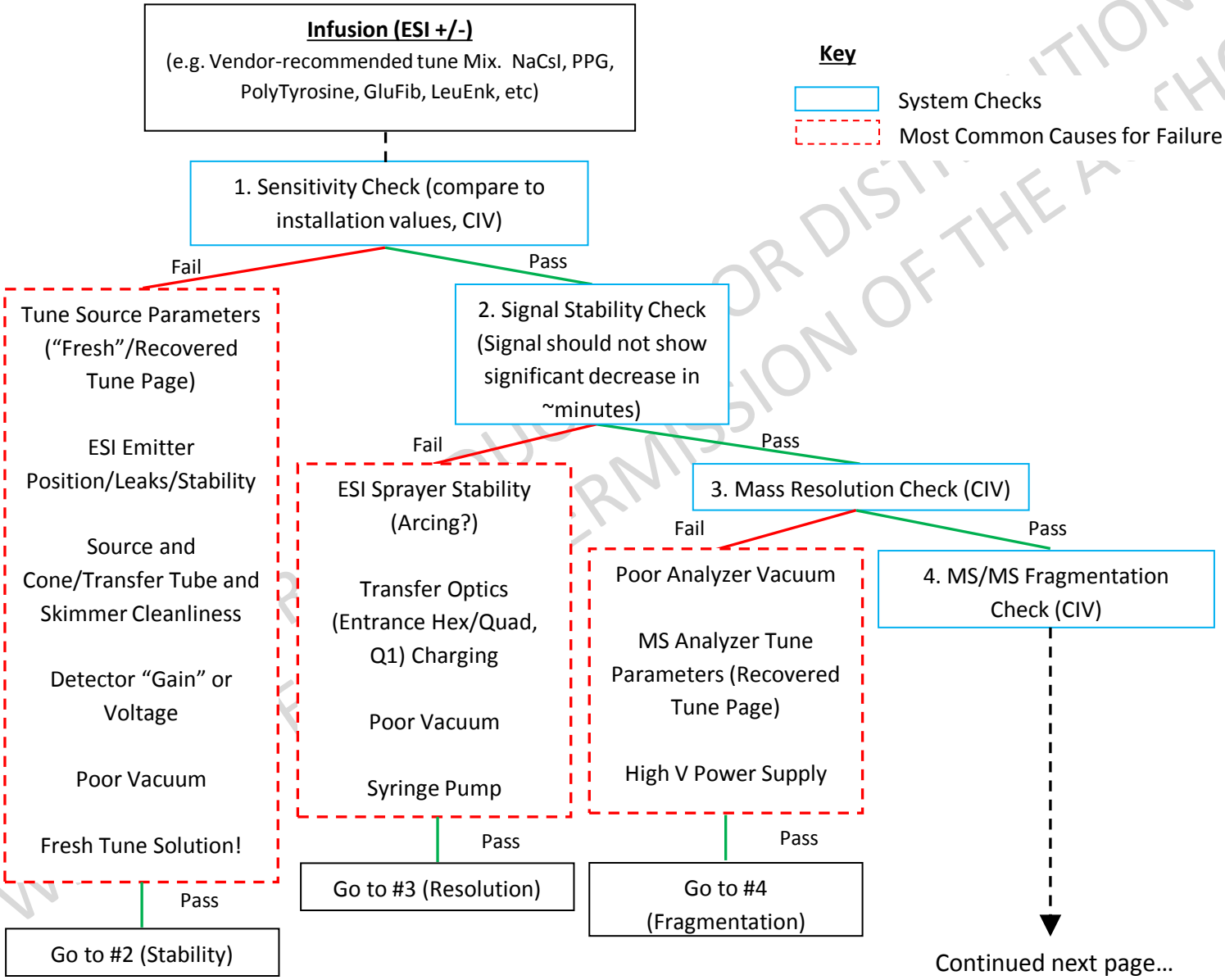


Columns & Hardware

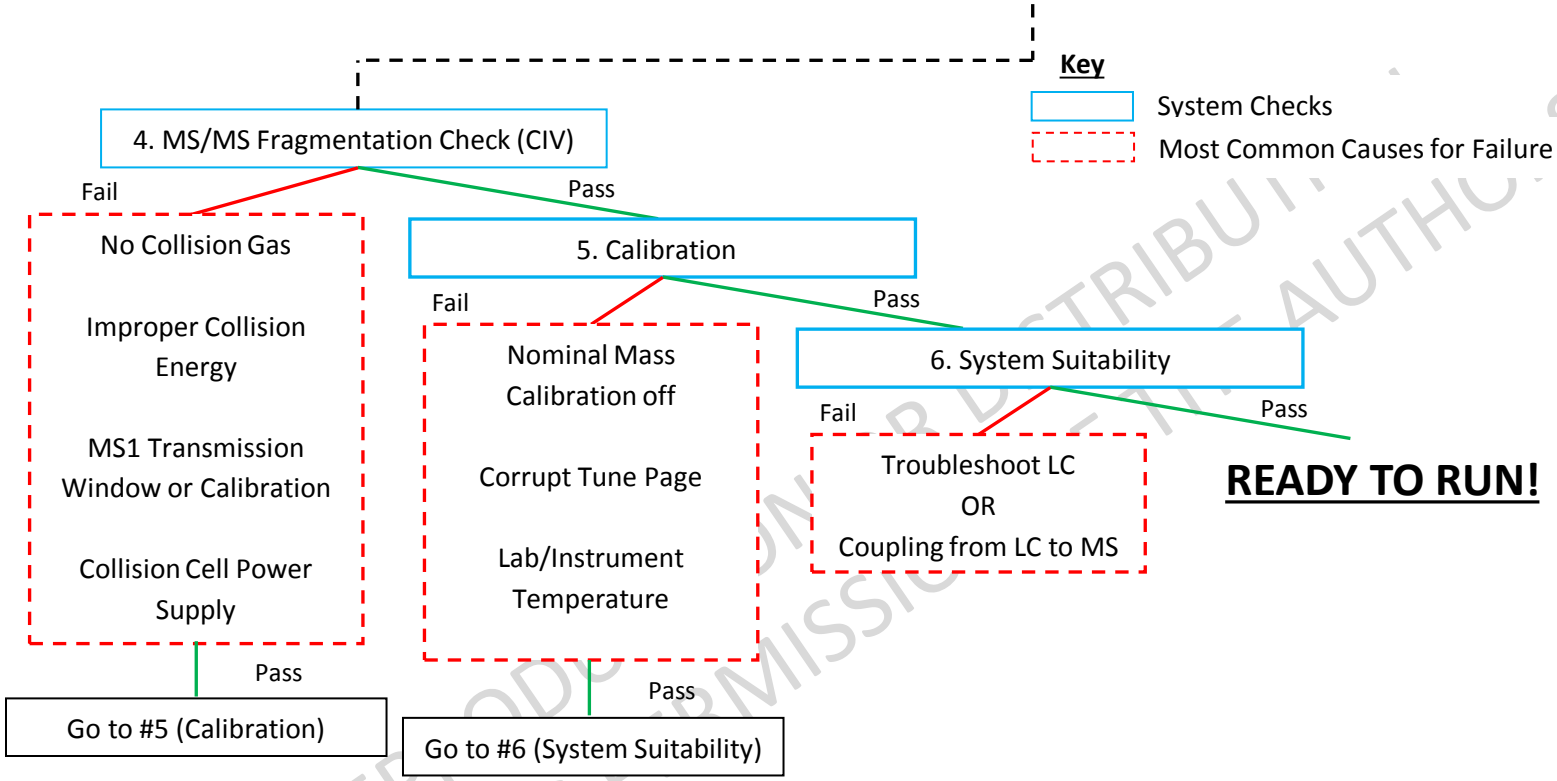


MS System: A Troubleshooting Workflow and Common Problems

MS System: A Troubleshooting Workflow



MS Troubleshooting Workflow, continued…



Remember VISUAL INSPECTION is key at all steps!
Can you think of other, less common failures for these system checks?

MS System: A Brief Word On Communications

Infusion (ESI +/-)
(e.g. Vendor-recommended tune Mix. NaCsI, PPG, PolyTyrosine, GluFib, LeuEnk, etc)

But what if I don't have PC to Instrument Communication?

Troubleshooting workflow assumes basic PC-to-Embedded PC Communication is Intact

Methods to Check for Communications

1. Basic Readbacks (should update regularly and have slight changes)
    1. Turbo Speed
    2. Vacuum Readings
    3. Power Supplies
2. Toggle Controls (watch for response)
    1. ESI Voltage on/off
    2. Other source parameters
    3. Collision Energy low/high if infusing

What to Do if No Response

1. If in doubt, reboot! (PC and Instrument embedded PC)
2. Check cable connections
3. Communication (ethernet) cards
4. Hard reboot of instrument electronics

Troubleshooting Typial Causes of Failure (Sensitivity)

Tune Source Parameters (“Fresh”/Recovered Tune Page)	Ion Transmission is drastically effected by source tuning parameters. Check values against installation values. If same, consider starting a ‘fresh’ tune page since tune files can become corrupt.
ESI Emitter Position/Leaks/Stability	Emitter position and spray stability is the both the easiest way to have a problem and to fix one. If possible, record the micrometer settings on your ESI position to make sure it is in the right place. If spray is sputtering, adjust voltage, gases. Consider cleaning or replacing sprayer components. Check for leaks. VISUAL inspection is key.
Source and Cone/Transfer Tube and Skimmer Cleanliness	Dirty skimmer and transfer tubes can kill sensitivity. Clean stainless steel components with sonication in 50/50 MeOH/water with 1% Formic acid (gentle) or 30% nitric acid (aggressive). Wash thoroughly.
Detector “Gain” or Voltage	Voltage or ‘gain’ effects all types of detectors, from EMT on QqQ, to MCP/ADC on Tof, to Electron Multiplier for automatic gain control (ion gating) on trapping instruments. Run manual or automatic gain checks.
Poor Vacuum	Mean free path dictates that MS systems need vacuum to operate, and incomplete vacuum will kill ion transmission. Check the vacuum gauges against the installation target values to be sure vacuum levels are appropriate for your mass analyzer.
Fresh Tune Solution	Probably the easiest way to fail a sensitivity check is to have an old tune solution, have the wrong organic composition, or have the pH wrong (add the acid!). Make it fresh!

Troubleshooting Typical Causes of Failure (Signal Instability)

ESI Sprayer Stability	Emitter position and spray stability is the both the easiest way to have a problem and to fix one. If possible, record the micrometer settings on your ESI position to make sure it is in the right place. If spray is sputtering, adjust voltage, gases. Consider cleaning or replacing sprayer components. Check for leaks. Check for arcing due to incorrect voltage or erosion of sprayer.
Transfer Optics (Entrance Hex/Quad/Q1 Charging)	Previous samples, mobile phase additives, and even vacuum oil mist can sometimes coat the optics just inside the source. These can be easily removed on most instruments (after venting). Clean according to manufacturers recommendations.
Poor Vacuum	Mean free path dictates that MS systems need vacuum to operate, and incomplete vacuum can cause signal instability. Check the vacuum gauges against the installation target values to be sure vacuum levels are appropriate and stable. An incorrect readback can be poor pumping, a leak, or a bad gauge.
Syringe Pump	Pulsation in the flow rate can cause signal instability. This is usually (but not always) at a slower frequency than the instability caused by an unstable electrospray. Check that the flow from the syringe (or other pump) is stable,

Troubleshooting Typical Causes of Failure (Resolution)

Poor Analyzer Vacuum	Inappropriate vacuum can cause poor resolution in both ToF, LIT, and OrbitTrap analyzers. Check the vacuum gauges against the installation target values to be sure vacuum levels are appropriate and stable.
MS Analyzer Tune Parameters (Recovered Tune Page)	Small changes in tune parameters can cause resolution issues. Load the backup tune page to make sure nothing odd has happened. If this fails, fall back to your hard copy records. Resolution is highly instrument-type specific, but are most often related to pusher and reflectron tuning for ToFs. Automatic gain control/space charging effects is a common cause of decreased resolution in trapping instruments.
High Voltage Power Supplie(s)	Check voltage readbacks on analyzer power supplies. If you have not recorded the appropriate values, call your vendor technical support and they typically can give you 'good' values over the phone.

Troubleshooting Typical Causes of Failure (MS/MS Fragmentation)

No Collision Gas	Check gas inlet pressure, and make sure collision gas is on in tune page. Check collision cell pressure is comparable to installation values (and should increase when turning collision gas on).
Improper Collision Energy (CE)	Check CE or normalized CE setting to be sure it is correct.
MS1 Transmission Window or Calibration	On instruments with a quadrupole as the first mass analyzer (QqQ, QToF, Q-Orbitrap), the MS1 transmission window and calibration is often ignored but is critical for precursor ion selection. Check MS1 calibration and transmission window using sliding window around infusion of known analyte.
Collision Cell Power Supply	Check voltage readbacks on analyzer power supplies. If you have not recorded the appropriate values, call your vendor technical support and they typically can give you 'good' values over the phone.

(Calibration)

Nominal Mass Calibration off	Remove the calibration (default cal) and check the nominal mass of a known standard. Follow instrument-specific instructions for correction of nominal mass value.
Corrupt Tune Page	Corrupt tune pages can cause calibration failures, manual and automated. Load a backup tune page or start a new tune page.

Preventive Maintenance

Simple things to keep your LC/MS running.

Typical PM steps

- Verify system performance prior to venting.
- Record vacuum readings.
- Vent the system following the recommended steps for your system. Always refer to the associated hardware manual.
- Service the vacuum pump(s).
- Replace the sprayer / probe electrode.
- Remove and clean the front interface assembly.
- Clean or replace air filters.
- Pump system down to base vacuum ~ 4 hrs.
- Tune and calibrate the system.

Venting a Mass Spectrometer

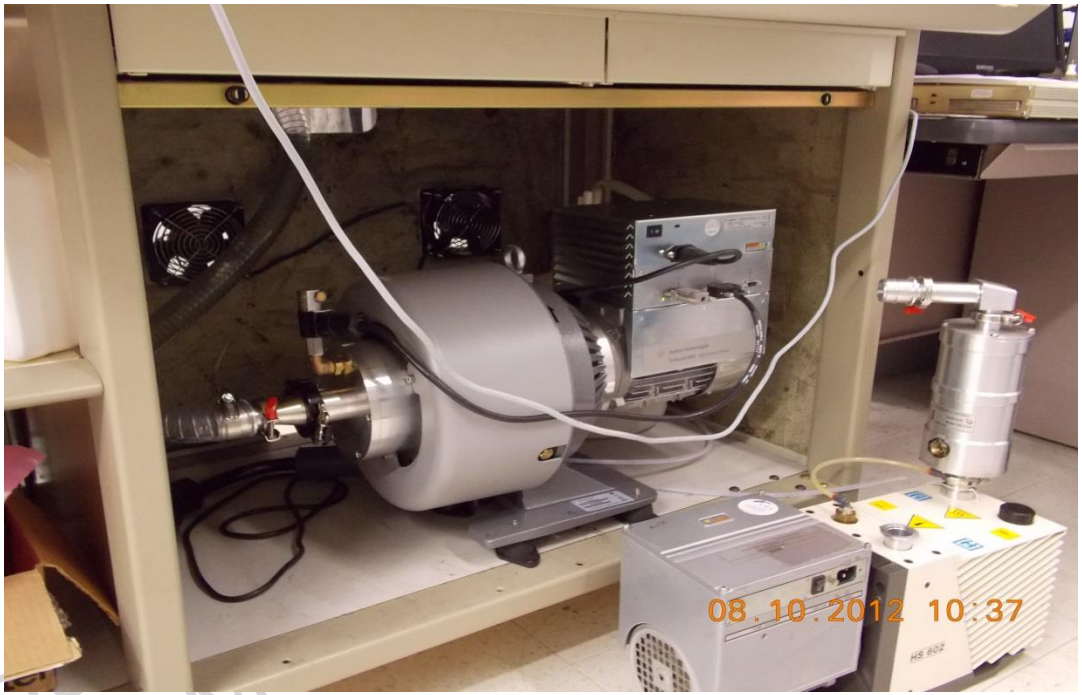
- It is very important to understand the procedures for venting your system.
- This is either accomplished through the software or by manually turning off switches.
- The turbo pump(s) should always be allowed to gradually spin down before turning off the rough pumps.
- Never break vacuum connections to force the venting cycle.

Vacuum Pump maintenance

- Oil change every 6 months or sooner depending on application and usage. Always follow manufacturers recommendation for type of oil.

- Scroll pump or Dry pump – Tip seal replacement every 18-24 months depending on pump model and manufacturer.

- Always inspect and clean the pump motor air intake to remove all build up of dust and dirt.



Ion Optics Cleaning

- Clean orifice, skimmer, interface region and associated optics.
- Recommended cleaning solutions – 10% formic acid, IPA, MeOH & H20 or Alconox & H20 – Sonicate 15-20 min.
- Blow off with dry N2 and inspect.

Ion Source - Probe / Sprayer Maintenance

- Clean / flush housing.
- Remove probe Assy. and replace capillary or electrode. Always refer to the manufacturers documentation for recommended adjustment positioning or spacing.
- Replace associated ferrules and fittings as necessary
- Inspect associated heaters for integrity and performance.
- Verify spray performance with standard tuning mix.