First tier troubleshooting:

1. Verify TIC – overlay with a known "good" TIC from a previous standard acquisition
    a. Lack of rich peaks (fewer)?
    b. Lower signal?
    c. => start troubleshooting
2. Verify spray current
    a. May be different names, chamber current etc.
    b. Too high – discharge
    c. Too low – no spray/flow, or bad power supply
3. Verify spray
    a. Visually inspect (with lens for nano) for droplets emitting from sprayer tip
    b. Verify sheath and counter flow for higher flow rate applications
4. Verify pump pressures
    a. Pay attention to composition (%B) relative to conditions
        i. Need to develop expected backpressures for your laboratory and specific conditions
    b. For H2O/ACN, initial conditions will have highest backpressure
    c. For H2O/MeOH, mid gradient will have the highest backpressure
    d. Is column heater working properly? May need a thermocouple to verify.
5. Are there adequate mobile phase volumes?
    a. Also, are the volumes updated in the software? Some apps prevent the pumps from running after a calculated volume has been dispensed.
    b. Test the pH of the mobile phase to ensure ion pairing agent is present

Second tier (problem still exists after checking all points from 1st tier):

1. Prime pumps at least 15 minutes
2. Check each fitting with a KimWipe for leaks – will be especially small on nanoflow.
3. Clean ion source (without compromising vacuum of mass spec)
4. Run a checktune solution to verify mass spec is OK
5. Run a known standard from a working instrument on the problematic LCMS (sanity check)
6. Verify collision gas pressures (is the tank empty?)