Agilent Technologies

Tips to Improve Signal-to-Noise Checkout

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Title: Tips to Improve the Signal-to-Noise Checkout

The following are some items to check that may improve the signal-to-noise level of the LC/MSD checkout.

1. Make sure that LC/MSD has been "steam cleaned" overnight. Steam cleaning is where you set the LC flow to 0.5 ml/min, nebulizer pressure to 60 psi, drying gas to 13 L/min, drying gas temp to 350 C, and if it is APCI, the vaporizer temp to 400C, and then make sure the MS stream selection valve is set to go to the "MS". Steam cleaning overnight has been shown to be one of the most important factors in improving the signal-to-noise level. The checkout column should be in line of the LC flow, so that the column is flushed and conditioned overnight also.

2. The checkout for sensitivity checking requires using 75:25 methanol water with 5 mM ammonium formate. Sometimes it is reported that adding 100 ul of formic acid improves the signal-to-noise. Depending on the condition of the ammonium formate, the pH may vary significantly. The addition of the formic acid will ensure the proper <7 pH.

3. Make sure the system is tuned properly, especially the mass axis calibration. If you set up the instrument on the first day and tune it at that time, make sure to tune it the following day after the quadrupole temperature has equilibrated. It takes approximately 11 hours for the quadrupole temperature to equilibrate. Changing quadrupole temperature will cause the mass assignment to shift up to 0.3 amu.

4. Perform the scan checkout first and check the background level in the TIC. On a good system with low background, the background level should be less than 200,000 counts. If it is above that level, and if you are not passing the signal-to-noise checkout, then try to determine the cause of high background and eliminate the background contamination. Select several spectra from the TIC and see if the spectra contain the same major mass peaks. If they do, then you have chemical contamination. Try to determine if the contamination is coming from the solvents, or from one of the LC modules, or from the column, or from the N2 gas, or from the spray chamber, and then eliminate the contamination. If the spectra are different, then the high background is not chemical contamination; is likely a nebulization problem, so check the nebulizer spray or adjust/replace the nebulizer needle.

If you are running APCI and you have a high background level in the TIC, check for the presence of masses 536 and 610. These contaminant ions come from the vaporizer insulator when it breaks. The 610 ion in particular will interfere with the 609.3 reserpine ion. If you see these ions, either bake out the vaporizer assembly overnight at 450 C or replace the vaporizer assembly.

In either APCI or electrospray, if you see mass 149 as a prominent mass in the spectrum, this indicates contamination in the water from red bloom algae. Make up new solvent, and make sure that the water is clean. If the water is coming from a filtration system, then the system should be flushed thoroughly before the water is taken.

5. Check the adjustment of the nebulizer needle.

6. The signal-to-noise macro generates and evaluates an EIC of the 609.3 ion with a +/- 0.2 amu window, so it is critical that the mass assignment is accurate. In addition to making sure the mass axis is calibrated properly in tune, the mass axis lag factors must be calibrated properly. The mass axis lag factors will affect the mass assignment in scan mode when you scan at a different A/D sampling rate compared to tune. To calibrate the mass axis lag factors, first make sure that the system is tuned, then on the command line type 'set_lag'. You can check to see if the mass assignment is off by looking at the spectrum of the reserpine peak in the scan mode checkout. The '609' mass should be at 609.3. If it is too far off from that value, then the mass axis lag factors should be recalibrated. If it is right on 609.3, then the mass axis lag factors are okay.

7. Make sure that the reserpine checkout sample is made fresh. You will start to loose response if the sample is over four hours old. If it was stored in a refrigerator, the intermediate dilution can be used to regenerate the final checkout sample.

8. If you suspect a bad checkout column, run the checkout without the column. The column has been specified to help achieve the required signal-to-noise level by eliminating or delaying the peak that would appear in the injections of the blank. If the column is in other ways causing problems with the checkout, then it can be bypassed.