PROBLEM
In order to assure the quantitative accuracy of LC-MS/MS systems and methods, validation studies must demonstrate freedom from any extraneous signals that augment analyte peak areas by more than 20% of the peak areas representing the lower limit of quantitation for each analyte (1). Such signals may result from samples (carryover and/or contamination) or from non-sample sources (contamination) by the analytes themselves or by other interferences having similar chromatographic and MS/MS attributes. Each source of extraneous signals and its contribution to quantitative inaccuracies must be revealed and mitigated.

METHOD INFORMATION
Examples involved various Thermo Scientific™ LC-MS/MS instruments, columns and software, some utilizing Thermo Scientific™ TurboFlow™ technology.

TROUBLESHOOTING STEPS

1. Distinguish between true analyte carryover versus other forms of contamination
   • Strategic injections of blanks and standards
     A Pre-Blank should show negligible carryover, 1st Post-Blank should show most, last Post-Blank should show least carryover. If all blanks show similar carryover then test contamination of mobile phase solvent(s) or blanks.

2. Apply systematic approach to reveal sources of carryover and/or contamination
   • Test mobile phase solvents for contamination
     Contamination of initial solvent can be revealed by doubling and tripling the column equilibration time before injecting the blank and observing that the contaminating peak(s) in the respective chromatograms double and triple accordingly.
   • Inject blanks after modifying LC-MS plumbing (bypass and/or swap components) to measure and rank carryover contributions of the autosampler, valves, connections, columns and MS source

TROUBLESHOOTING STEPS

• Suspect #3: Valve(s)
  Worn and dirty rotor seals cause carryover. Clean or replace them. In some cases, alternative materials for rotor seals and stators reduce carryover. Poor drainage of injector valve waste line can cause carryover.
  3. Repair or replace defective components that contribute to carryover
  4. Modify autosampler and/or LC parameters, especially chemistry

OUTCOMES
1. Follow good practices (3) to prevent contamination
2. Follow preventive-maintenance procedures to avoid or minimize wear that can lead to carryover
3. Implement changes to the system and method that have been proven to minimize carryover

SUMMARY
Contamination must be distinguished from carryover. Identify and remedy its sources.
Carryover can result from spatial pockets (tiny cracks, channels or gaps) or surfaces that hold residual analytes. Identify and remedy its sources. Hydrogen bonding, ionic, metallic and hydrophobic interactions between analytes and surfaces can be quenched by adding competitive inhibitors to the mobile phases and washes and/or by changing the chemistry of the surfaces.

REFERENCES

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