Carryover & Contamination Causes & Cures - Assuring the Quantitative Accuracy of LC-MS/MS Methods

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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.
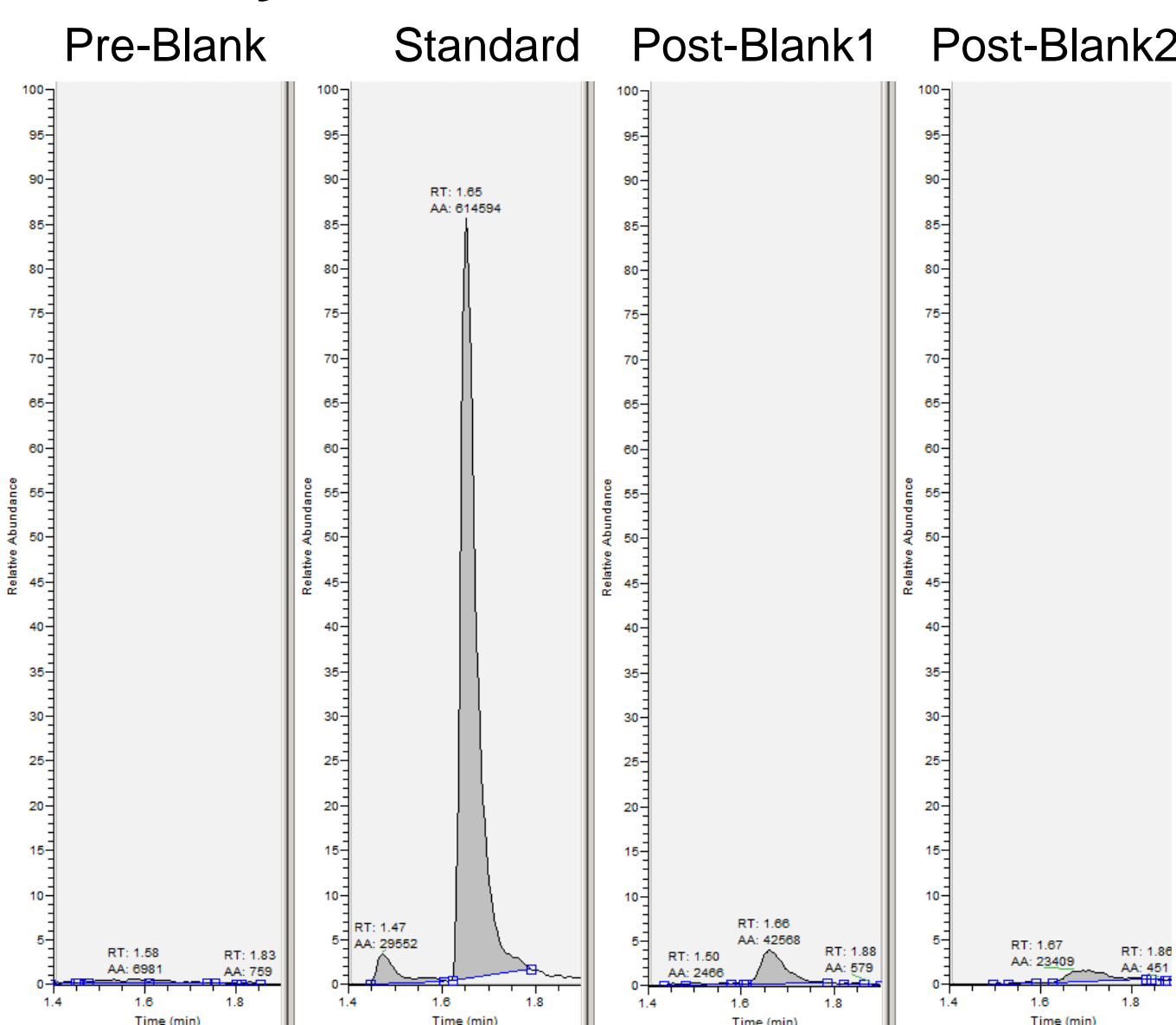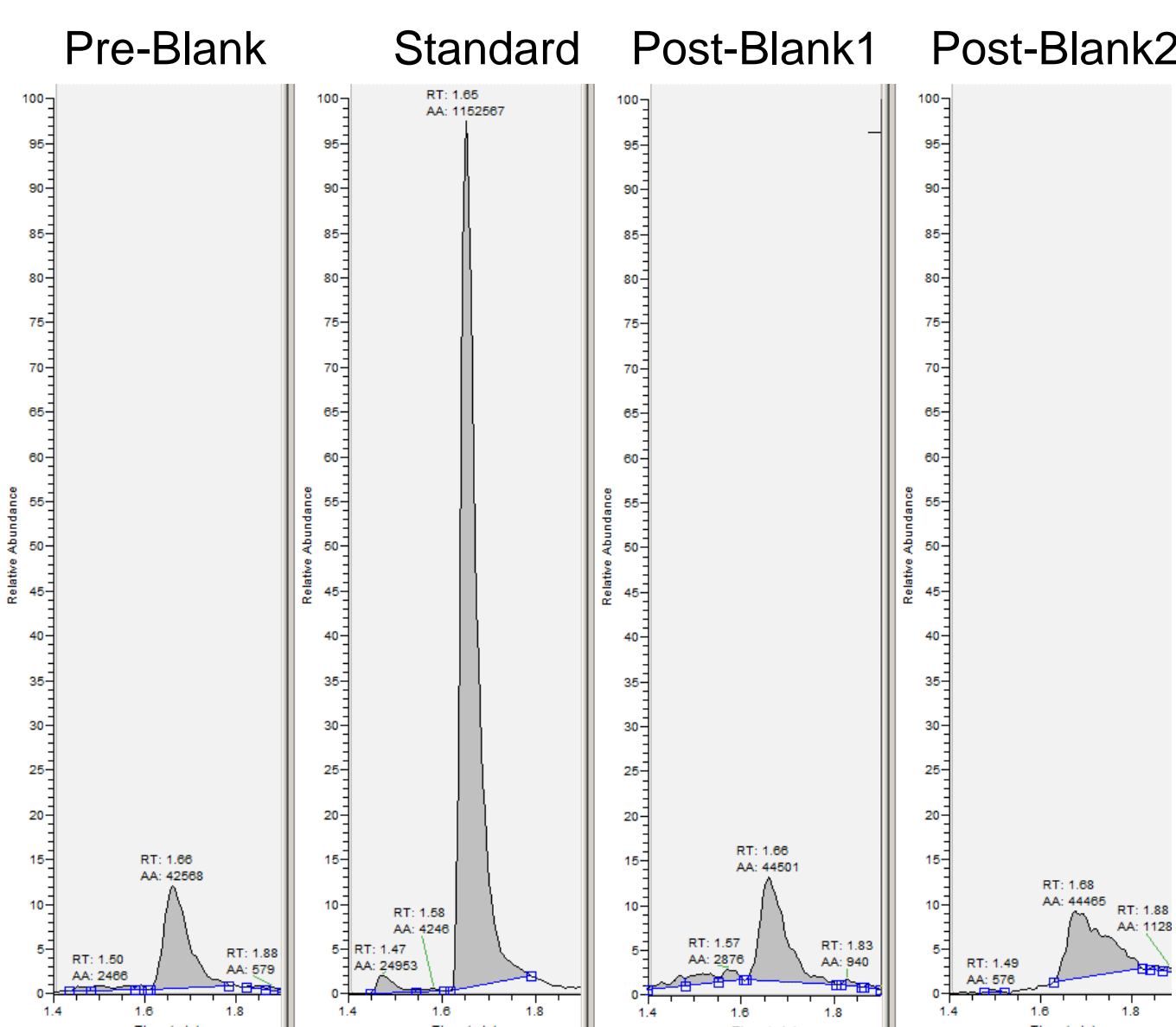
Figure 1. Carryover

Pre-Blank   Standard   Post-Blank1   Post-Blank2

Figure 2. Contamination

Pre-Blank   Standard   Post-Blank1   Post-Blank2

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

Figure 3. TurboFlow LC-MS/MS components
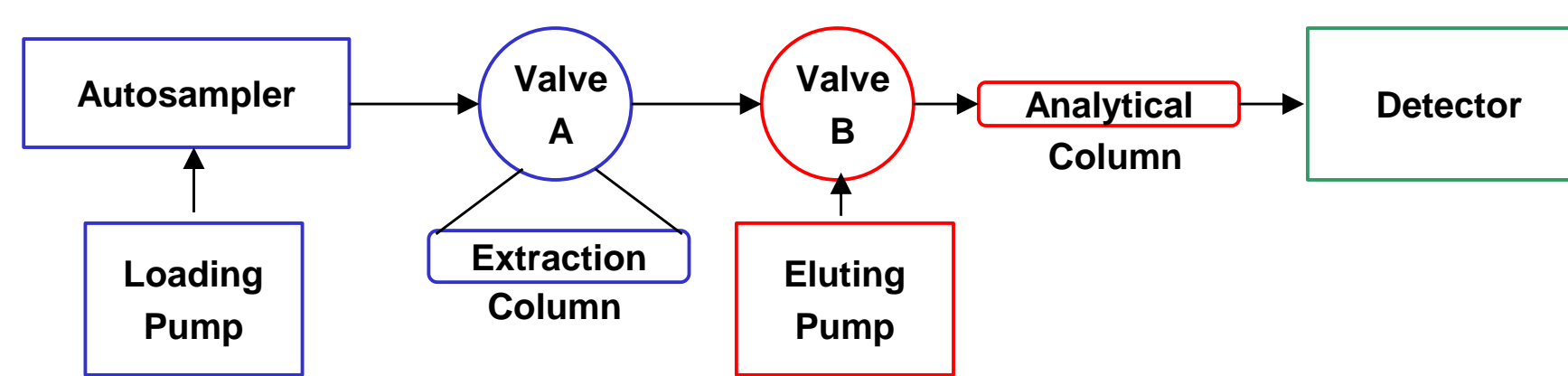
- Suspect #1: Autosampler
  - Sample syringe plunger worn?
  - Wash solvents flowing?
  - Wash solvents strong enough?
  - Needle penetration into sample too deep?
  - Needle guides, wash stations contaminated?

Figure 4. Specimen contamination by prior specimen in filled deep-well via deep needle penetration
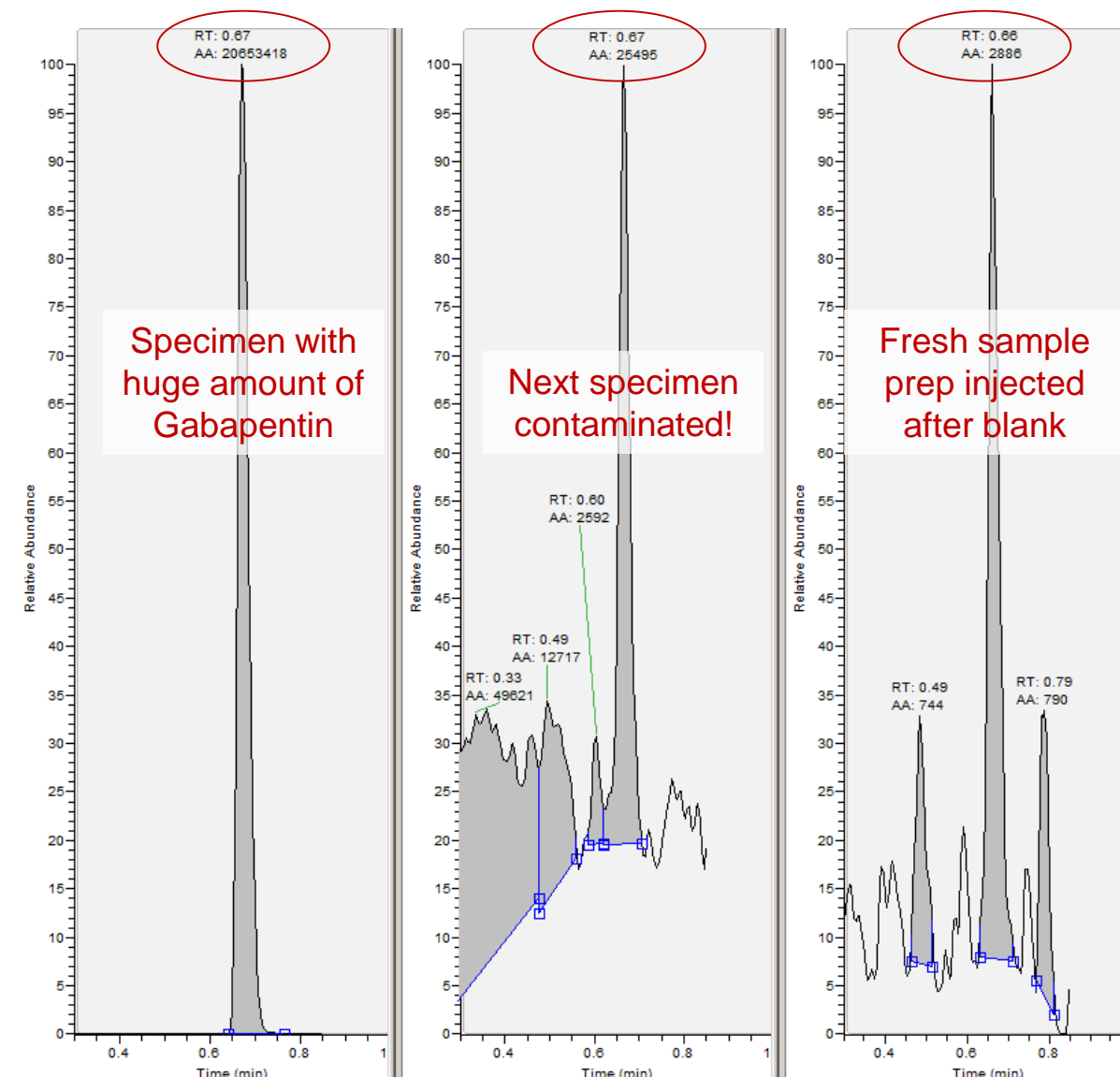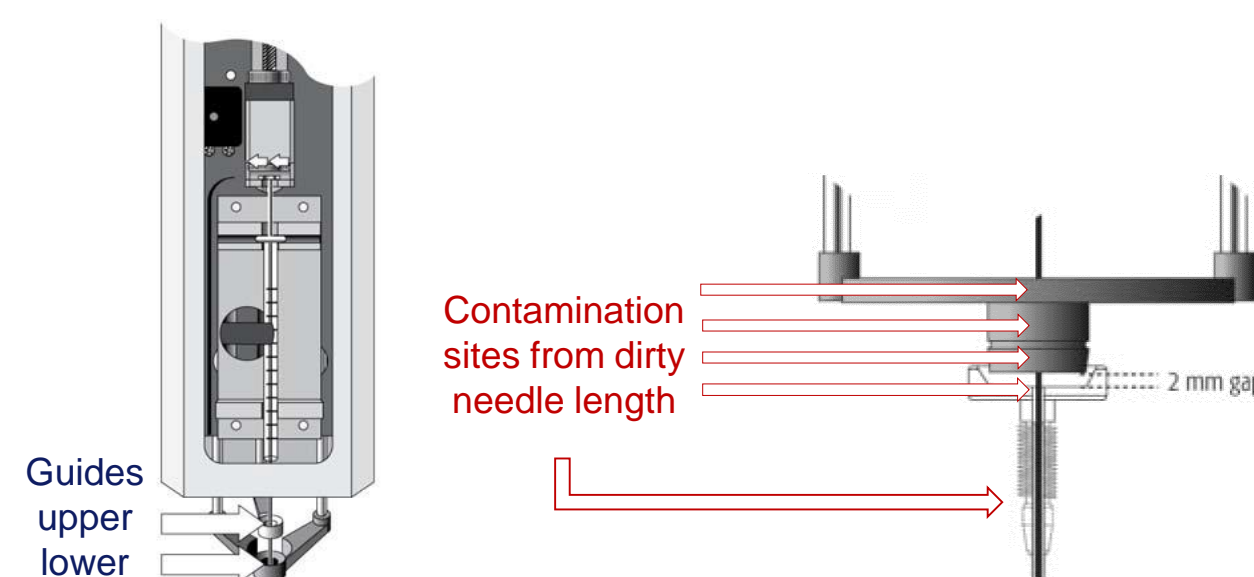

Figure 5. CTC syringe needle guides

Figure 6. Example autosampler method

Wash 1: H2O + 0.1% formic acid or disodium-EDTA + 2% acetonitrile (anti-microbial)
Wash 2: "Magic Mix" 40% acetonitrile + 40% isopropanol + 20% acetone
Addition of trifluoroethanol reportedly prevents carryover of peptides & proteins (2)

- Suspect #2: Column(s)

Run Pre-Blank and Standard, then STOP. Replace column with new one. Run Post-Blank. If carryover appears, improve column washes in LC method.

Figure 7. Example column washes

Rapid switching between aqueous and organic solvents clean large particles in TurboFlow column, Magic Mix from Loading Pump cleans HPLC column and Valves A & B.

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

Figure 8. Insignificant carryover

Pre-Blank   Standard   Post-Blank1   Post-Blank2
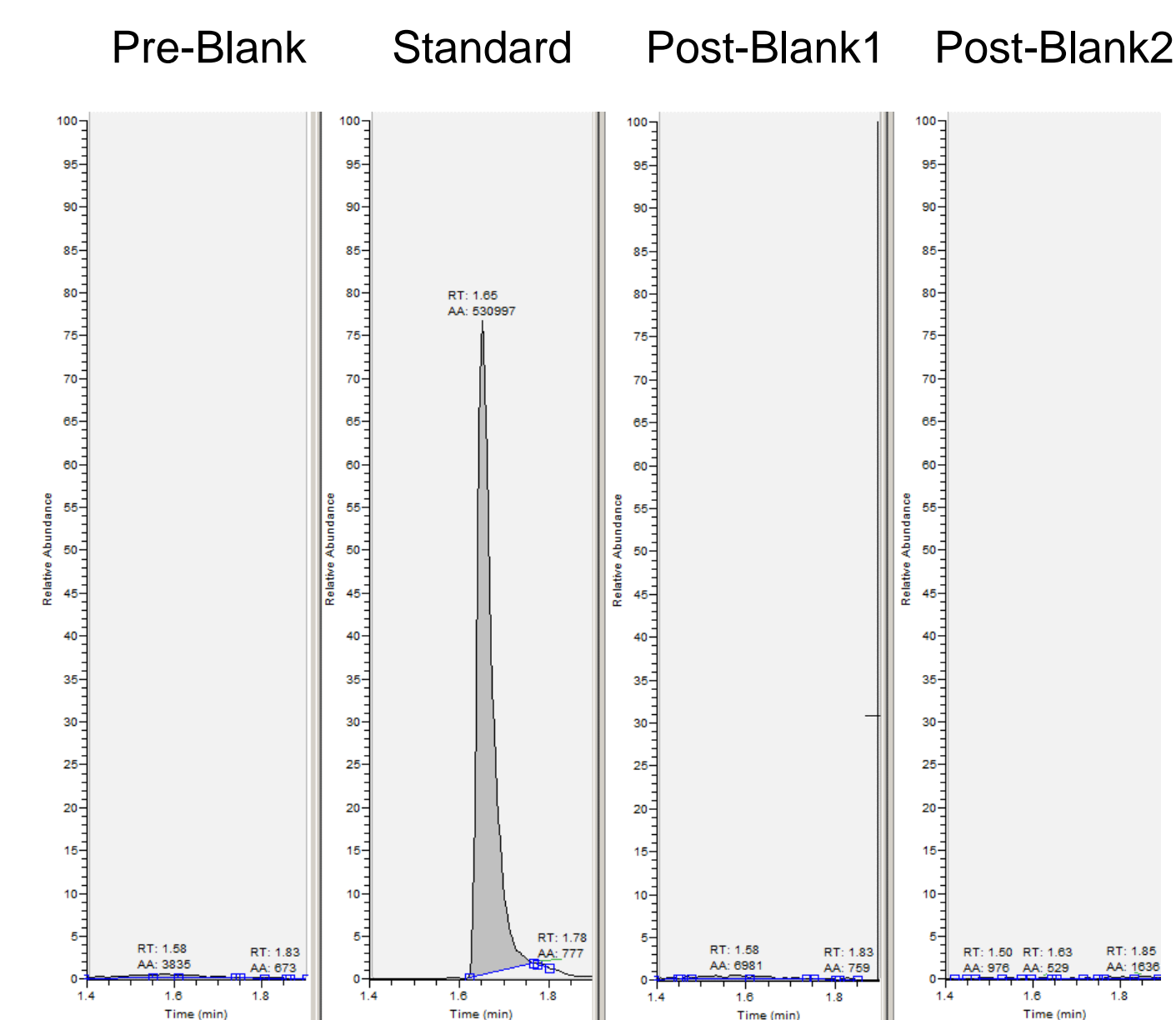
SUMMARY

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

1. N.C. Hughes, E.Y.K. Wong, J. Fan & N. Bajaj, Determination of Carryover and Contamination for Mass Spectrometry-Based Chromatographic Assays, The AAPS Journal 2007; 9 (3):E353-E360
2. G. Mitulovic, C. Stingl, I. Steinmacher, O. Hudecz, J. Hutchins, J-M. Peters & K. Mechtler, Preventing Carryover of Peptides and Proteins in Nano LC-MS Separataions. Anal. Chem. 2009, 81:5955-5960.
3. J. Di Bussolo, B. Kennedy & E. Goucher, Good Practices for Successful High-Throughput LC-MS Bioanalysis. MSACL 2017 Poster B11.

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