

TROUBLESHOOTING DETERIORATING PEAK SHAPE

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PROBLEM

The SST samples at the beginning of the run look good. The start of the run – all peaks look good. Partway through the run, the peak shape has deteriorated.

METHOD INFORMATION

- Serum samples extracted with MtBE using Supported Liquid Extract
- Shimadzu LC/AB Sciex MSMS with MPX-2 stream LC multiplexing, problem only on one stream
- MPA: 2mM Amm Acetate in H₂O
- MPB: 2mM Amm Acetate in MeOH
- 100 x 3 mm, 2.6µm C18 column, with guard cartridge
- Column oven 55 °C
- 50µL injection volume
- Lots of other assays run on this system!

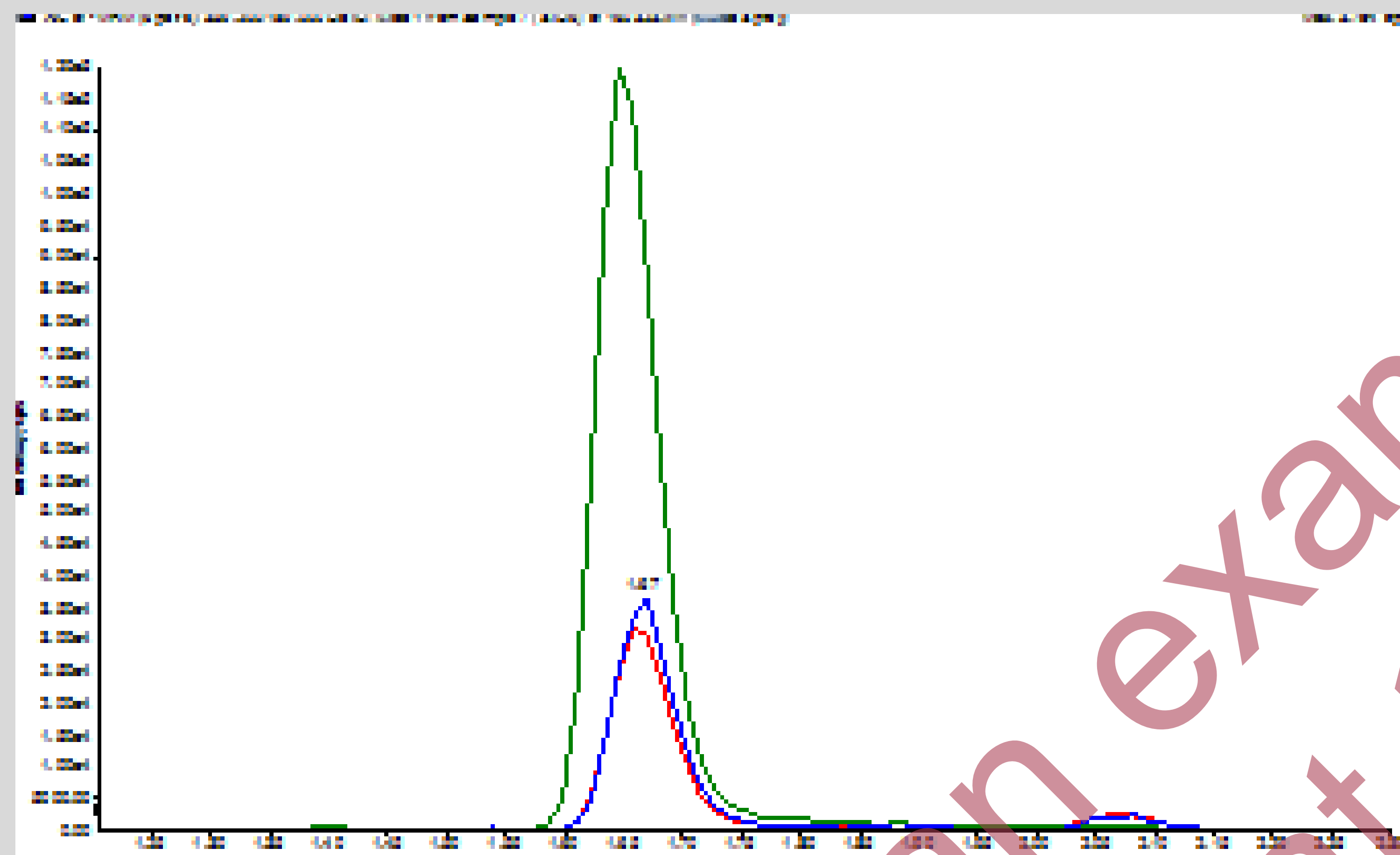


Figure 1: Good Chromatogram at beginning of run

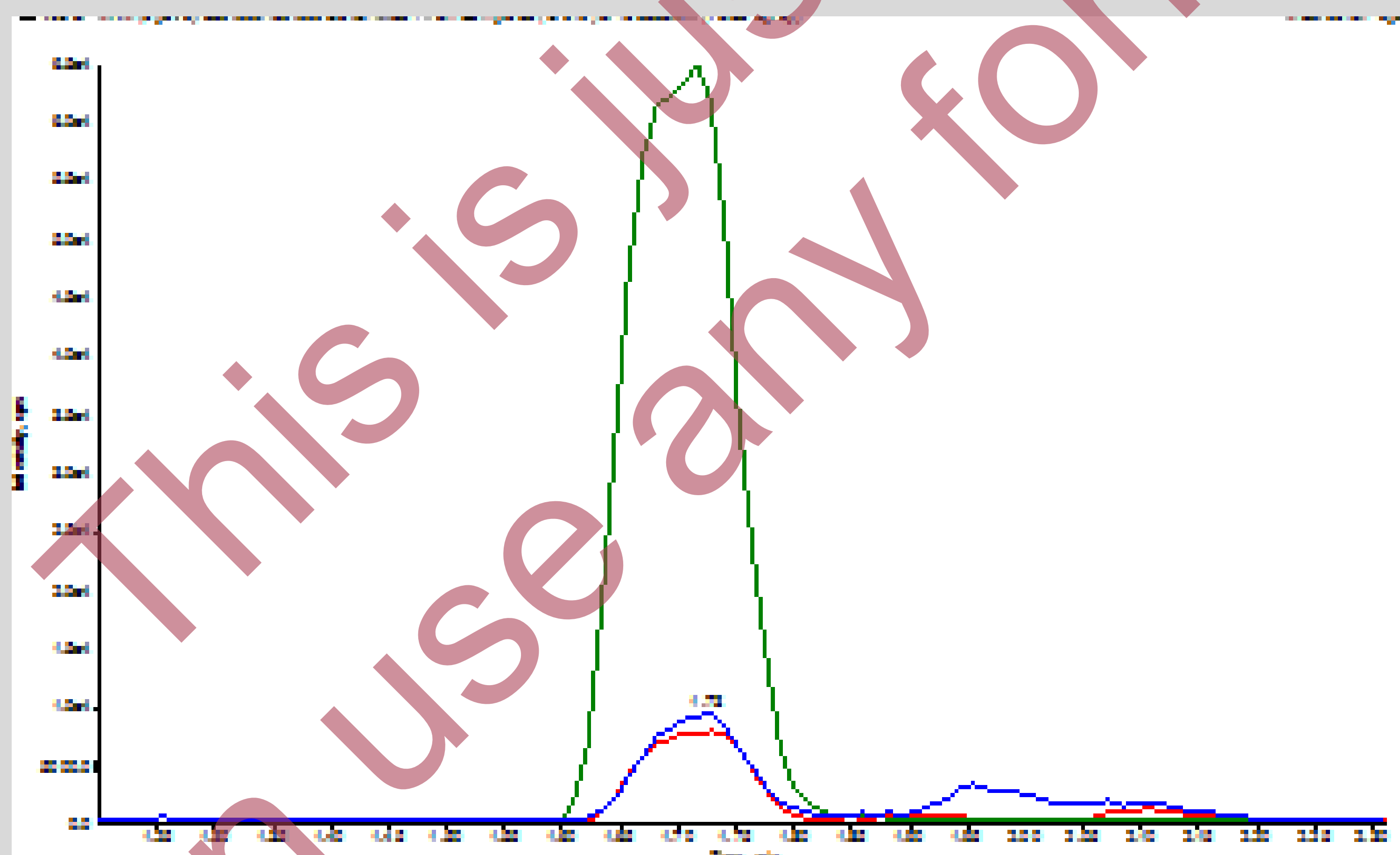


Figure 2: Chromatogram with obvious peak shape issues towards end of run

TROUBLESHOOTING STEPS TAKEN

First, let's think about it:

- 1) Sample Prep, LC, or Mass Spec?
 - Clearly it's an LC problem!
- 2) Why would peak shape deteriorate?
 - column problem like a void in the column or particulate stuck on frit?
 - could it be the guard cartridge?

Now, let's try to fix it!

- Tested the column on another LC to confirm the problem is the column
- Backflushed column with 95:5 Acetonitrile:Water for approximately 30 minutes
- Reconnect column to original LC and inject SST samples

OUTCOME

The chromatograms are back to normal, backflushing the column fixed the problem!