

## LC Troubleshooting Series

### Introduction

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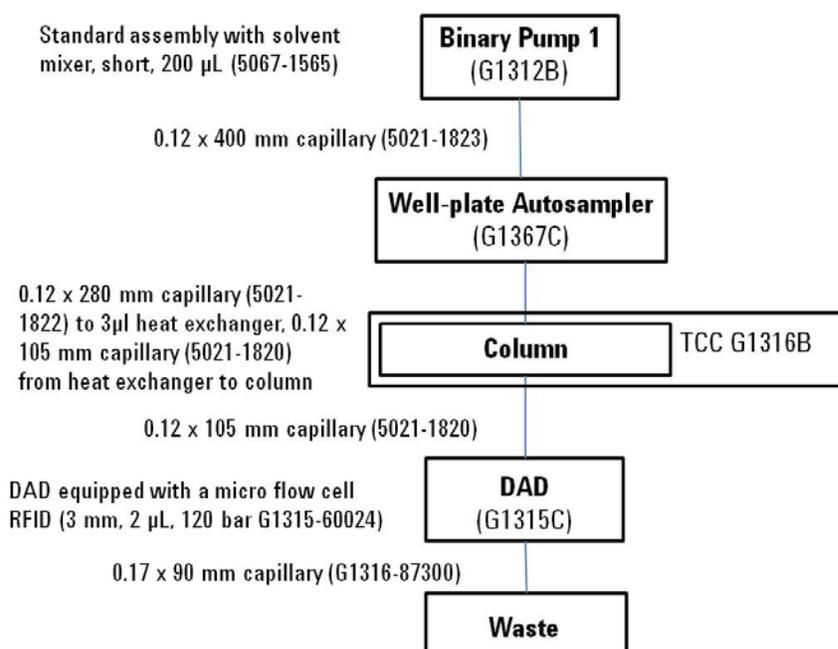
There are three ways to look at LC Troubleshooting:

- The Instrument
- The Column
- The Method

### Tip for Troubleshooting the System

A big part of troubleshooting is determining where the issue is coming from by looking at all possible symptoms and eliminating unlikely causes.

The key LC instrument parts to consider when troubleshooting are: Autosampler (Injector), Pump, Detector, Data System or Integrator



Draw up a system map, making note of the capillaries used to connect components, and the way your instrument is stacked.

## Tip for Troubleshooting Your Column

Tip: Run a baseline chromatogram when you get a new column. You can use the same test mix that was used on the QC test chromatogram that comes with your column. Or you can use a standard you keep on hand, or your usual method.

It is best to use isocratic conditions.

Over time, comparing this chromatogram to your current results will help you identify whether the column is losing efficiency, or causing an issue.

## Tips for Determining the Source of Your Issue

You'll want to narrow your issue to one of the following:

- System backpressure: is it too high, too low or erratic?
- Changes in chromatographic resolution, specifically retention time, selectivity or efficiency
- Problems with peak shapes – ghost peaks, tailing peaks, split peaks, fronting peaks
- Baseline noise or baseline drift – often this is associated with gradient methods or detector problems

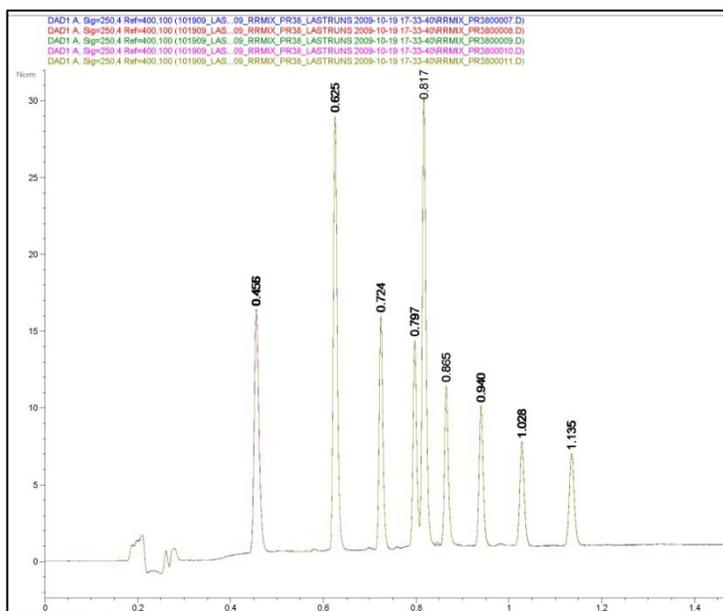
## Things to think about:

- If there is an instrument logbook, check it for recent entries that might hold clues.
- Has someone else been using the system?
- Has the instrument recently been modified or serviced?
- Has the method been modified by someone? (check revision dates)

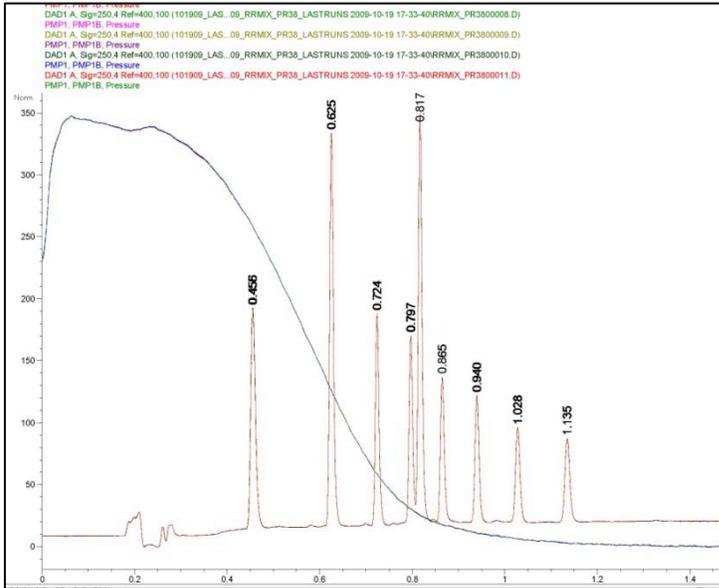
## Using the System Pressure Trace

Another important troubleshooting tool on newer systems is the system pressure trace. View your system pressure trace to evaluate trends and find clues to the source of an issue.

Here's an example of a "perfect" 5 chromatogram overlay:



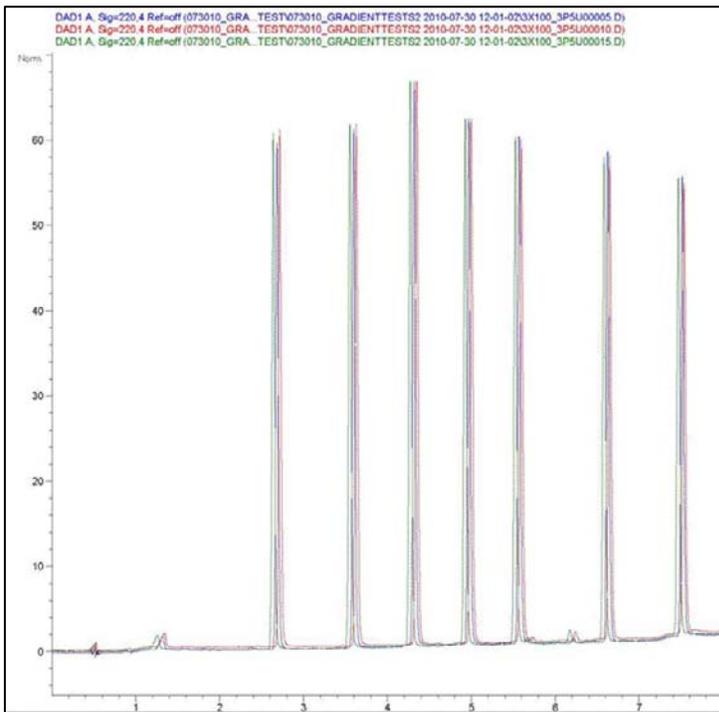
And here's the same image with the pressure trace overlay:



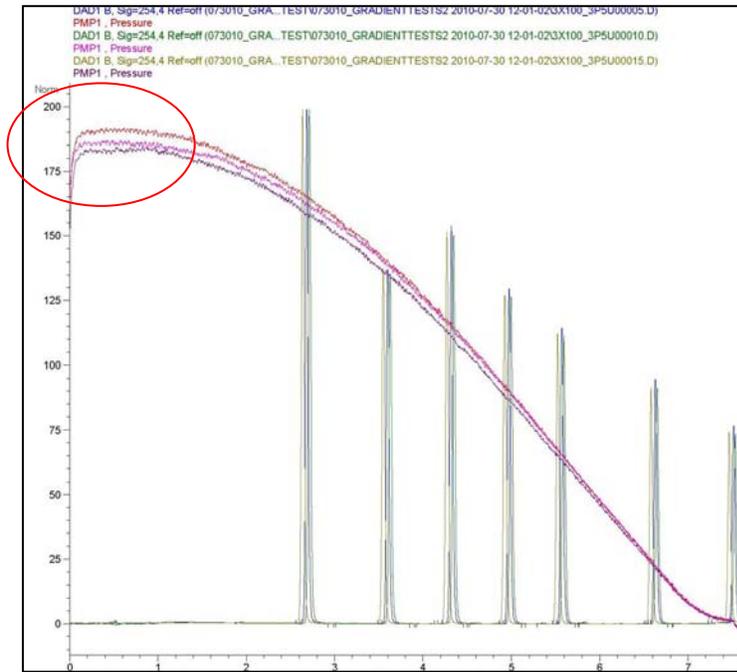
See in this image that the pressure trace is identical in each run, as expressed in one solid line, in the same way the chromatogram looks like one chromatogram, even though it is actually five runs.

The pressure trace at first shows the system re-pressurizing from the sample injection, then relatively constant, and finally trending to lower pressure as the gradient program introduces solvent with a lower

After some time, it began to run like this:



In this series of chromatograms, we see shifting retention, which indicates there is an issue. Retention time variation can be a result of pump malfunction, unstable column temperature, leaks in the system or inadequate run to run equilibration, to name a few of the most common reasons. How should we start – rebuild the pump, change the column or make new mobile phase? No! We should look for all the available evidence that might support which problem it might be.



Looking at the pressure trace, overlaid here, helped identify the source of the issue as being in the autosampler, because pressure changes occurred at the start of each run. Ultimately, the issue was narrowed down to a leaking needle seat.

### Other Notes about the Videos:

When viewed on Agilent.com, there is some key functionality that helps you get the most all videos:

- You can jump to just the section you need to see, by clicking on the outline that is features on the right hand side of the video.
- All videos have downloadable notes that can be accessed through the button at the right hand lower corner of the player. These notes will include the chromatograms and exhibits we discuss in the videos.

If you have additional questions at any time, please visit us on the web at [www.agilent.com/chem/contactus](http://www.agilent.com/chem/contactus) to connect with an Agilent technical support representative.