LC Troubleshooting Series
Autosamplers and Injections

Mike Woodman is an Applications Chemist, specializing in Agilent instrumentation.

Bill Long is a Senior Applications Scientist for the Chemistries and Supplies Division.

Autosamplers and injection methods can be a source of trouble if they are not used correctly.

Look to your autosampler specifications to help with understanding operating parameters. You can search for them on Agilent.com

Routinely monitoring the pressure trace on your instrument is also a good way to keep an eye on your autosampler /injector performance.

How Autosamplers Work

There are two main types of autosampler design: fixed loop and variable volume. Most newer instruments, including the Agilent 1260 and Agilent 1290, have variable volume injectors.

Peaks Too Small

If your peaks are too small:

- Check your sample volume to ensure you have enough in the vial to reach the needle
- Check valves and metering syringes for leaks

To validate autosampler accuracy, install a tared vial and inject a volume that is conveniently verified gravimetrically.

For manual injections, if peaks are too small, check the needle port for leaks.

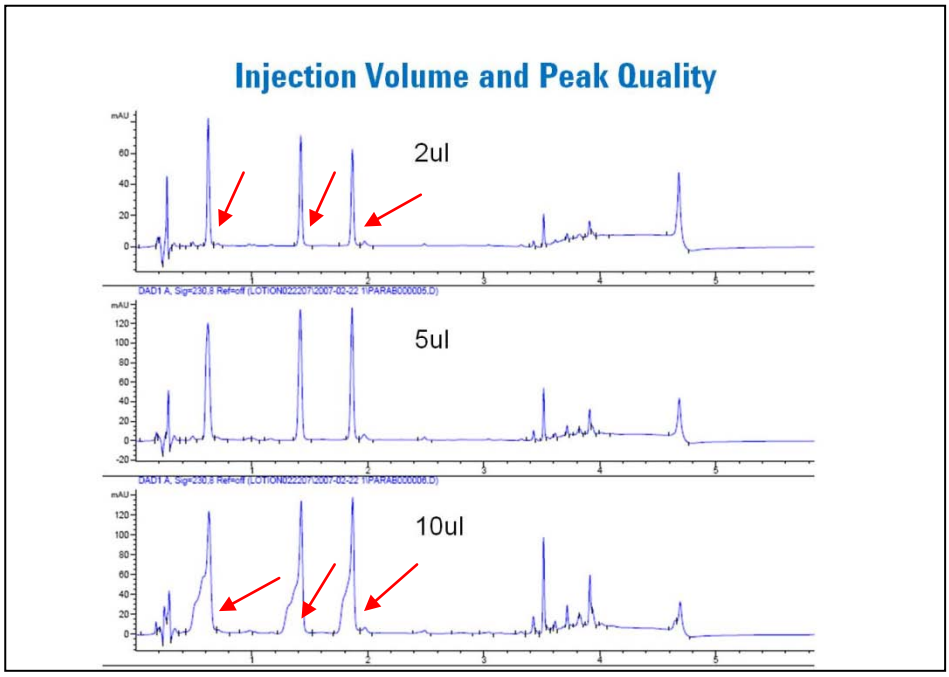
Peaks Too Large

The autosampler is not likely to be the cause of peaks that are too large, unless the metering heads are the incorrect size.

Poor Reproducibility with Repeat Injections

Injection volume affects peak width.

The pressure trace is a good place to look to evaluate issues with reproducibility. You can analyze trends in pressure changes, and narrow potential sources.



Injection volume will affect peak width, as we discuss in the Peak Broadening segment of the LC Troubleshooting Video Series. The example to the left shows injections at 2 µl, 5 µl and 10 µl to demonstrate this concept. Note the various differences in peak width on peaks 2-4.

Non-Linear Calibration Curves

2 ways to create a calibration curve:

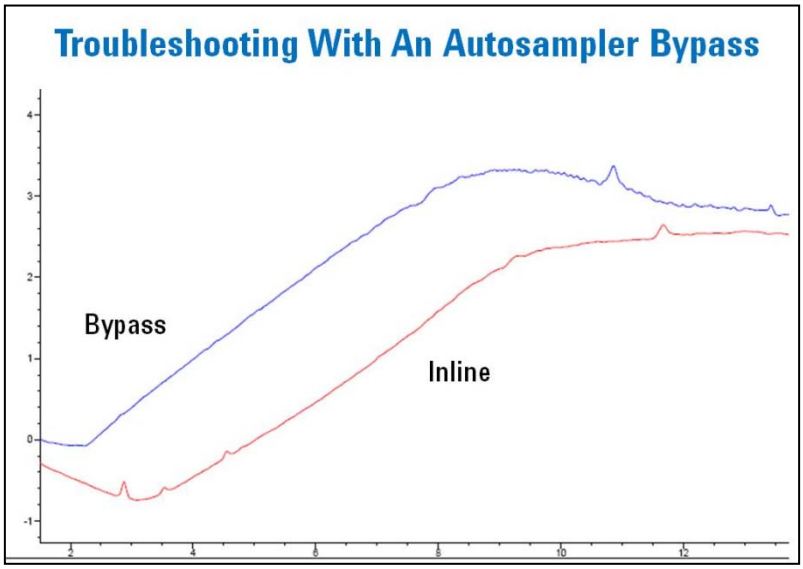
- Use standards of different concentration introduced with the same injection volume
- Make one concentration and let the autosampler inject different volumes.

Most linearity problems come from the detectors response range. If you have a linearity problem in both area and height, look at detector conditions.

Extra Peaks

Autosamplers are a good place to look if you are getting extra peaks. Contamination in the needle port or needle seat from other samples can cause ghost peaks.

Extra peaks may also come from the mobile phase. You can bypass the autosampler and run a blank gradient to see if this solves your ghost peak issue. If it does, you know the source is the autosampler. If it does not, then look to the mobile phase and other areas.



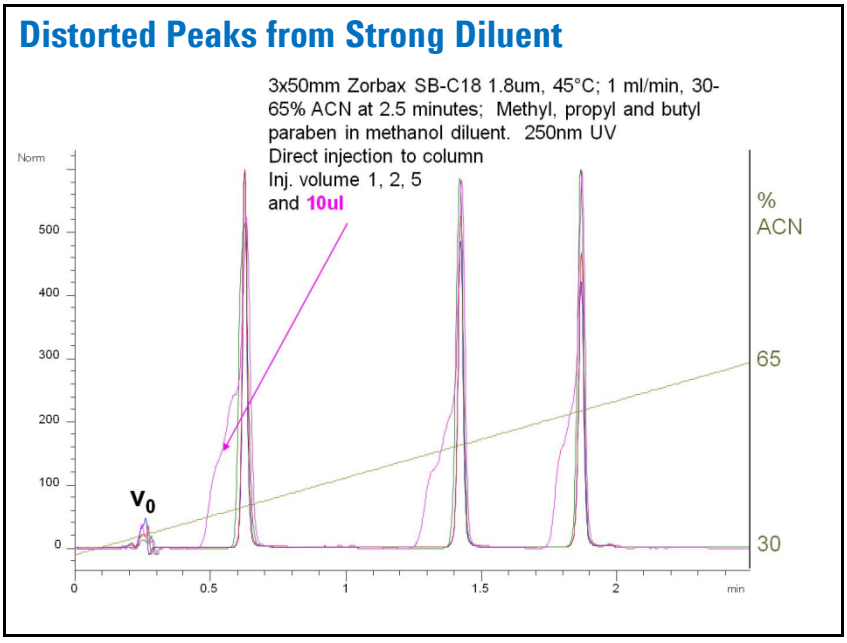
Extra peaks in the chromatogram, particularly with gradient analysis, may also be coming from the mobile phase. In the most extreme of examples of troubleshooting, we have bypassed the autosampler by connecting the pump directly to the column and running a blank gradient.

Changing Peak Shape

Changing peak shape can be an indication of a blockage or worn component within the autosampler.

The sample diluent is also something to consider. The diluent has the potential to upset the equilibration of the column and cause peak distortion.

If the diluent is a strong organic or if the diluent is an aqueous solution with a very different pH or molarity from the mobile phase, and especially if the injection volume is relatively large, we can easily end up with distorted peaks, as you see in this example to the right.



Summary

Tips to prevent and/or address issues with your autosampler or injection:

- Be familiar with your autosampler specifications and behavior, and understand the limitations of manual injections.

- Use your system's pressure trace to help you identify trouble and isolate the source, if possible

- For issues with extra peaks, run a bypass of the autosampler to confirm whether the source is there. If it is, look to wash key components that come into contact with the sample.

- If you have a linearity problem in both area and height, look at detector conditions.

- Remember that strong diluents in the sample can upset the equilibration of your column.