Agilent Technologies

Chromatographic Troubleshooting
Peak Shape Problems
General

A15603

Peak symptoms

No peaks

This is usually due to operator error; possibilities include injection on the wrong column, incorrect signal assignment, attenuation too high (peaks are present but not visible), a bent syringe needle in an automatic sampler, etc. Check system parameters for the analysis.

Inverted peaks

This is likely an inappropriate signal assignment definition (e.g., B - A with sample injected on column A) or incorrect polarity with a TCD.

Extra peaks

These are divided into two classes: **Additiona**l peaks appear on the chart in addition to those expected from the sample. **Ghost** peaks appear even when no sample is injected (and also appear among the genuine peaks during a sample run).

1. Peaks appear during a blank run:

   These are ghost peaks, usually found during temperature•programmedruns; the cause is contaminants trapped at the head of the column at the relatively cool starting temperature. These are released and chromatographed as column temperature rises.

   Ghost peaks are often observed when a column has been at the starting temperature for some time. For example, the first few runs in the morning often contain ghost peaks.

   - Ghost peaks may arise from septum bleed, carrier gas impurities, and contamination in plumbing by oils, grease, and other materials. Less commonly, they may be caused by reaction of

stationary phase with trace levels of O_2, H_2O, and/or other materials present in the carrier gas.

- A contaminated inlet may also produce ghost peaks. Residues in the inlet are volatilized or pyrolyzed and swept onto the head of the column. Try reducing inlet temperature; if this eliminates or reduces ghosts, the inlet should be cleaned.

2. Additional peaks appear when pure sample is injected:

   - These might be ghost peaks as described above. Make a blank run; if the peaks persist, they are not sample related.

   - A common cause of extra peaks, assuming the sample is pure, is degradation of one or more components by an overheated inlet. Test this by reducing inlet temperature.

     Operate the inlet at as low a temperature as possible without causing peak broadening due to slow vaporization. Also, perhaps a more volatile solvent can be used. In extreme cases, derivatize the sample before analysis.

   - Metal columns may also degrade the sample. Extra peaks in this case are usually broader than their immediate neighbors since they are generated along the entire length of the column. If this is the cause, changing to an all•glasssystem may be necessary.

Deformed peaks

The ideal peak, rarely occurring in chromatography, is a pure Gaussian shape. In practice, some asymmetry is always present, particularly near the baseline.

1. The peak rises normally, then drops sharply to baseline:

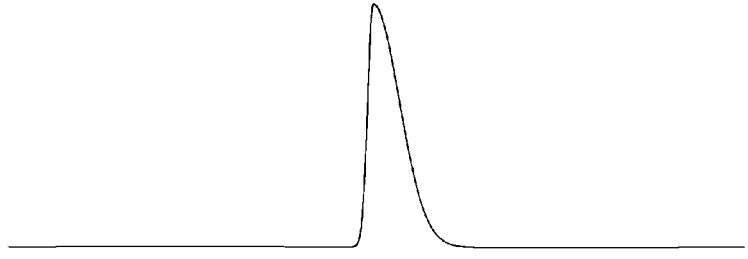
Figure 9-1.

Overloaded Peak

- The most likely cause is column overload; dilute the sample ten•foldand run it again.
- This may also be two (or more) closely merged (unresolved) peaks; lower oven temperature 30°C and repeat the analysis. If partial separation is seen, merged peaks are present.

2. The peak rises sharply and then falls normally to baseline:

Figure 9-2.

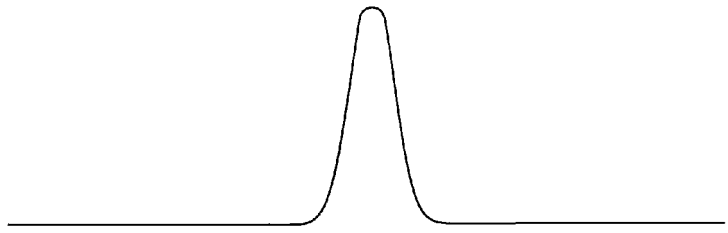


Abnormal Interaction with Column Material

- Interaction with column material is a frequent cause. Silanized support may help. An all•glasssystem may be required if metal column tubing is the source.
- Column overload with a gas sample often shows this effect; try injecting less.
- This may be a merged peak situation: Running at lower (30°C) oven temperature will increase resolution, perhaps enough to reveal merged peaks.
- Low inlet temperature may cause this, as can poor injection technique.

3. Top (apex) of the peak is deformed:

Figure 9-3.



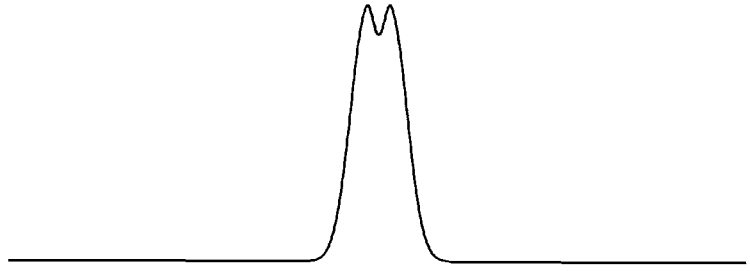
Detector Overload

- Detector overload is the probable cause: The distortion is not easily seen on the chart but reveals itself in an integrator report. In extreme cases, doubling amount injected causes little or no increase in peak size.

  Inject less sample, or dilute it, whichever is more convenient (dilution is usually the best approach). Since the detector is at the upper limit of its response, substantial dilution (100 times or more) is needed to be well within normal operating range of the detector.

4. Top (apex) of the peak is split:

Figure 9-4.



FID/NPD Flameout, or TCD with H_2 (in He Carrier)

- Verify that this is not a merged peak situation: Reduce oven temperature 30°C and repeat the run. If the **split** peak becomes better resolved, it is probably a merged pair.
- Gross overload of an FID may cause the top of the peak to invert, giving appearance of a split peak. Check gas flows; overload is more likely when flows are too low.

  Dilute the sample by a factor of at least 10 and repeat the run: If the split disappears, overload is the problem. It is advisable to dilute samples even more, by 100 or 1000, to ensure the detector is not close to its overload condition. Such dilution generally improves linearity as well.
- H_2 peaks, analyzed with a TCD using He carrier, often shows a split top.