

Gas Chromatography Problem Solving and Troubleshooting

Question:

Sometimes I experience problems in obtaining a straight line when generating a five-point calibration curve. What causes this problem?

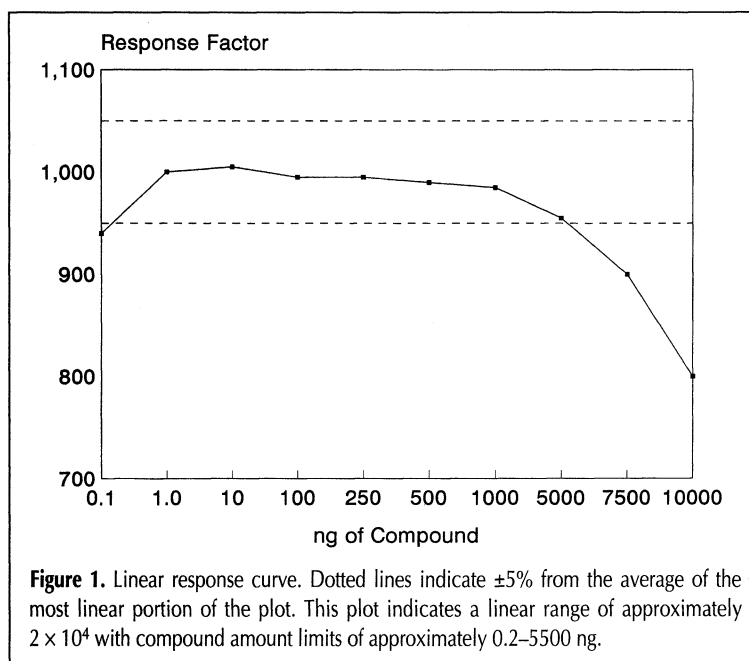
Answer:

There are a number of reasons for nonlinear response curves. The first area of investigation should be the samples. The assumption that the samples cannot be responsible for the problem is often made, especially if the onset of the nonlinearity problem was sudden. However, errors in preparation, degradation, evaporation, and contamination are a few possible causes of nonlinearity. Sometimes only a few of the samples or compounds may experience this type of problem, which encourages the incorrect conclusion that the samples cannot be responsible for the problem. Preparing another set of samples or using another gas chromatograph (GC) are the easiest methods to determine if a sample is responsible. Be aware that injection techniques or parameters can significantly affect linear behavior; extra care needs to be exercised to avoid these types of errors when using another GC.

Injection-related problems should also be investigated if it is determined that the samples are not the source of the nonlinearity. Any changes in the injection process can alter the amount of sample entering the column. These changes include injection volume (injection volume is not linear [1]), sample solvent, syringe technique, type of liner, split ratio, column position in the injector, septum purge flow rate, and injector temperature. Differences or changes in any of these conditions between the individual samples used to generate the five-point curve may cause one or more points to deviate from linear behavior. In addition, only one or a few of the compounds in the sample may exhibit nonlinear behavior with a change in the injector or injection technique. Capillary split injectors are probably the most sensitive to changes, so special care is necessary when using this type of injector.

The detector is another potential source of nonlinear calibration curves. A linear response from a detector occurs over a range of compound amounts or concentrations. If some or all of the compound amounts fall outside of this range, a nonlinear calibration curve is obtained. The linear range for most detectors is 10^4 – 10^5 . This means that a linear response is obtained for samples with 10,000–100,000 times difference in their concentrations. The detectors with the worst linear ranges are the electron-capture detector (ECD) (10^2 – 10^3) and the flame photometric detector (FPD) in the sulfur mode, which is nonlinear for most compounds.

The linear range does not provide any information concerning the actual compound amounts that comprise the end points of the range. Although the range of compound concentrations may be smaller than the detector's linear range, any compound concentrations outside the range cause a deviation



The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC-MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward to JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting*, P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

Dean Rood
Associate Editor

from the linear response curve. The sample concentrations have to fall within the linear range of the detector for the response to be linear. The linear range and its end points are often different for various compounds and detectors, which means they usually have to be determined experimentally. One method to determine the linear range is to inject a series of samples containing compounds over a broad concentration range. The compound concentrations should be slightly higher or lower than any of the anticipated samples, and a minimum of five concentrations between the end points should be used. Generally, a sample at or slightly below the minimal detectable level should be the lowest level sample. Plotting the results is the easiest method to visualize and determine the linear range. Figure 1 illustrates one plotting method that uses response factors (peak area divided by sample amount). The response factor is fairly constant within the linear range of the detector; it begins to deviate as the compound concentration moves beyond the extremes of the detector's linear range. With this type of plot, it is easy to see when a compound concentration falls outside the linear range of the detector. A deviation of less than 5% from the linear range is usually acceptable.

There are several other causes of nonlinear behavior. Thermal conductivity, photo-ionization, electron-capture, flame photometric, and mass spectrometric detectors are concentration-dependent. This means that their response is dependent on the flow rate of gas through the detector. Thus, changing a carrier or makeup (auxiliary) gas flow rate or type or temperature program (because carrier gas flow rate is temperature dependent) can alter detector response. The effects on compounds and concentrations are not always the same. Another possible cause is related to the characteristics of selective detectors. These types of detectors do not respond to every compound eluting from the column. It is possible that a nonresponding compound is eluting at or close to the same time as a responding compound. The nonresponding compound may affect the response of the detector. This is common with FPD and nitrogen-phosphorus detectors and may be a cause of nonlinear behavior, especially for compounds in a sample matrix (e.g., soil, blood). Finally, active compounds (primarily those containing an -OH or -NH group) may be adsorbed by the column or injector liner, which reduces the amount of compound that reaches the detector. In general, a compound at a lower concentration is more affected than the same compound at a higher concentration, and this often results in nonlinear calibration curves.

Reference

1. D. Rood. Troubleshooting. *J. Chromatogr. Sci.* **33**: 87 (1995).