HPLC Flow Rate and Sensitivity

Typical detectors in HPLC such as UV-Vis, fluorescence, and refractometer detectors yield responses proportional to the amount of detected molecules in the flow cell at a given time. The higher the concentration in the cell, the higher the response will be. Mass spectrometers behave like concentration sensitive detectors depending on the ion source (including electrospray over their typical flow rate ranges).

Since the peak area is established by capturing the signal over time, the longer period the same amount remains detected in the flow cell, the greater the peak area response will be. And because the time spent in the flow cell is inversely proportional to the flow rate, decreasing the flow rate will lead to a proportional increase in the area response (half the flow rate will equate to double the peak area).

However, because sensitivity is proportional to the peak height and not the peak area, decreasing the flow rate will not increase sensitivity unless the peak height is also increased. Therefore, if decreasing the flow rate leads to a loss in efficiency/peak broadening, the peak height and subsequent sensitivity may decrease even if the peak area increases. Conversely, increasing the flow rate may lead to an increase in sensitivity if the peak shape sharpens and yields a greater peak height even with a decrease in overall peak area.

During method development, it is only useful to adjust the flow rate through a range where the efficiency is not compromised, and it is usually not feasible for flow rate gradients or changes within the time period of eluting peaks that need to be quantified. As such, flow rate is typically not a useful parameter to consider when significant increases in sensitivity are required, but can be a moderately useful tool for minor increases.

Common situations where the peak area response correlation to flow rate needs to be critically considered, are methods development on columns having a very wide range in practical linear velocities, (such as monolithic type technologies) and column dimension scaling.