Why Is My Peak Area Reducing With Flow Rate?

Chromatography Today Help Desk

We had an interesting issue come into the Helpdesk this month from a customer running clinical samples. The question they posed was why does altering my flow rate have an effect on the sensitivity of my UV detector? The observation was that as the flow rate increased the peak area started to decrease in accordance with Table 1

Table 1. Effect of flow rate on a concentration sensitive detector

Flow Rate / mL / min	Area
0.2	1166.1
0.6	388.7
0.8	291.5
1.0	233.2
1.2	194.3
1.4	166.6
2.0	116.6

If this data is plotted out in graphical format and a curve fitted through the data we start to see something quite interesting occur (Figure 1).

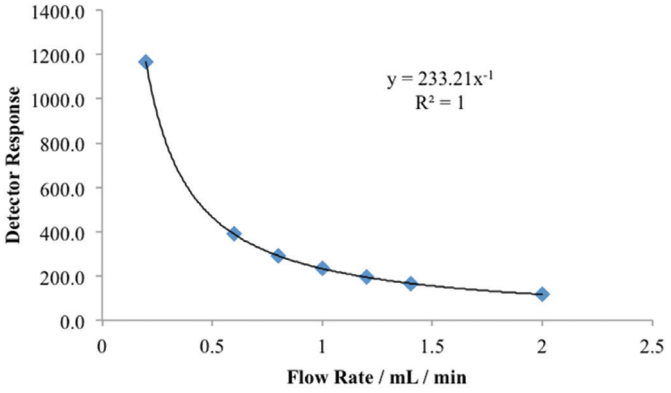

Figure 1. The effect of varying the flow rate on the detector response

This is an interesting issue and one that newcomers to chromatography often struggle with when first using UV detection or another concentration sensitive detector. So what is happening, why is the response going down with the flow rate?

First we have to make some sensible assumptions before we can get behind the mathematics of this. The first assumption is that the same mass is injected on all occasions; if this is the case then the following starts to apply;

We will assume a UV detector and consider what happens with the absorption characteristics of the detector, noting though that the same principle is applicable to other concentration sensitive detectors and this includes mass spectrometers under certain flow conditions.

When we use a concentration detector to monitor the response, then the detector will generate a specific peak height dependent on the concentration within the detector at the point in time. So if we assume that we can generate a perfect square peak, so the peak width will increase as the flow rate is reduced and consequently so will the peak area, this is shown in Figure 2.

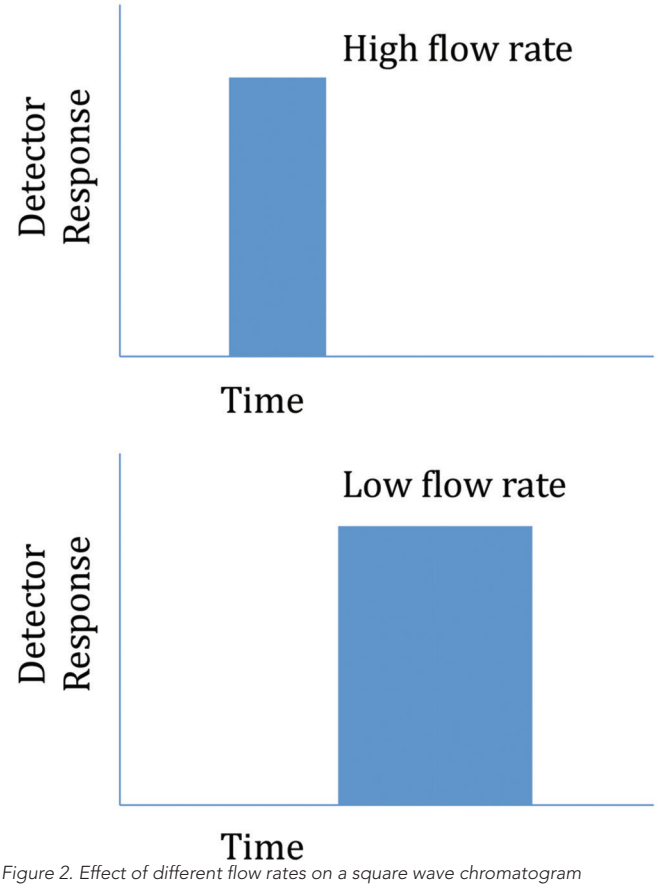

Figure 2. Effect of different flow rates on a square wave chromatogram

It is still important to understand what the relationship is between the flow rate and the area. For this a little mathematics is required.

$$A_i = \frac{k_i m_i}{Q}$$

where

A_i – is the area response from the detector to compound i

k_i – is the detector response factor (sensitivity of detector to compound i)

m_i – mass of compound i injected
Q – flow rate

Thus increasing the flow rate will result in a decrease in the response from the detector.
For a mass sensitive detector such as an evaporative light scattering detector, the above does not apply. In general mass sensitive detectors tend to be destructive detectors, and are often affected by the elution solvent parameters and consequently will give a finite signal dependent on the total number of interactions that occur with the molecule of interest and the detector. An easy way to determine if a detector is mass sensitive or concentration sensitive is to stop the flow when the compound of interest is eluting, with a concentration sensitive detector there is no change in the detector response, but for a mass sensitive detector the response will drop to zero very quickly as the compound being analysed is effectively destroyed.

This then raises an interesting point, what about a mass spectrometer coupled to a HPLC system. This is clearly a destructive detector and yet it can be classified as a concentration sensitive detector. How is this possible? We will come back to this in a later edition, but for readers who are interested, articles by Gallagher [1], and also he article by Klink [2] are recommended reading. In both of these articles the following experimentally derived data is presented and demonstrates the advantages of running at lower flow rates (Figure 3).

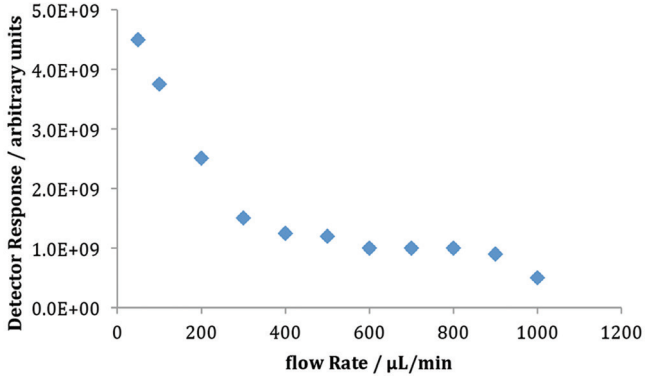

Figure 3. Effect of flow rate on the response with ESI (adapted with permission from [1]. Copyright (2003) American Chemical Society.)

It should also be noted that as smaller i.d. columns are used there is inherently an increase in the sensitivity, which arises from the following equation.

$$Response = \frac{mN^{1/2}}{(2\pi)^{1/2}V_0(1+k)}$$

where the response is dependent on the absolute mass of sample loaded onto the column (m), N is the system efficiency, k is the capacity factor and V_0 is the column dead volume which is clearly related to the column diameter. It should be noted that by keeping the mass loaded onto the column constant, whilst reducing the column id will lead to overloading the column (see Figure 4) which will in turn affect the efficiency.

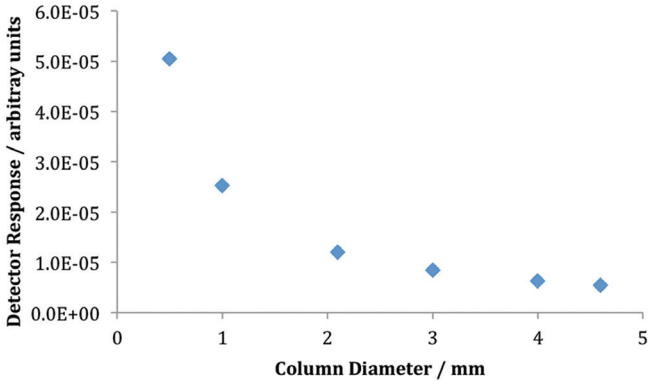

Figure 4. Effect of varying the column diameter and hence void volume of the column on the detector response

Overall it is important to be aware of both the effect of the column id and also the effect that the flow rate can have in terms of the sensitivity of an assay. It is also important to be aware that the injection volume should be scaled in accordance with the column diameter to avoid overloading the column, which will result in a worse peak shape. This in turn will affect the sensitivity of the assay. Finally, it should also be noted that dead volume of the chromatographic system can also have an effect on the peak shape, and consequently the sensitivity. We have shown that flow rate can have a profound effect on analyte peak shape and assay result. Therefore care should be taken that the optimisation of the chromatographic parameters are made in conjunction with the optimisation of the detector settings.

References

[1] Gallagher, RT, Balogh MP, Davey, P, Jackson MR, Sinclair, I, Southern, LJ, Anal. Chem. 75 (2003) 973-977

[2] Klink, F, http://www.sepscience.com/Information/Archive/MS-Solutions/232-/Frederick-Klink