Analysts often need to make calculations related to their chromatographic systems. Yet, many do not know what calculations are appropriate, or they use incorrect formulas that can cause serious errors. A good understanding of how to measure and calculate various working parameters from an instrument and a chromatogram does not require strong theoretical knowledge. Some high-school algebra and the willingness to make a little extra effort are the only prerequisites for improving the accuracy and usefulness of common chromatographic measurements. Calculating and applying these measurements correctly facilitates instrument and method maintenance. Effective method transfer from instrument to instrument requires a good working knowledge of chromatographic computations as well.

In this “GC Connections” column, I’ll present a number of useful measurements and calculations, based on a test chromatogram, that can help gas chromatography (GC) users to better understand the analytical process and thereby improve control over the quality of their results.

The Chromatogram
The classic elution chromatogram is a two-dimensional representation of the amount of solute exiting the column as a function of elapsed time since the separation experiment began. In GC, this plot represents detector response during the period after solute injection. Many of the parameters that chromatographers must understand derive from measurements of retention time and peak width made from the chromatogram. These measured and calculated values and their trends over time are excellent diagnostic tools that indicate how well a GC system functions and point to developing problems before they become serious.

Unretained peak measurements: Figure 1 illustrates a simple chromatogram with three peaks. The first peak is the detector response to substances that are not retained in the stationary phase. These components traverse the column entirely in the mobile phase, as expressed in the designation of their retention time ($t_M$, in which the subscripted M indicates its non-retained characteristic). If one of the non-retained solutes evokes a detector response, then the chromatogram will include a peak at this time. A thermal conductivity detector will respond to air in a sample, but a flame ionization detector will not. To measure the unretained peak time, analysts purposely inject an unretained substance to which the detector responds. Among these substances are methane or butane for flame ionization detectors, air for thermal conductivity detectors and methylene chloride vapour for electron-capture detectors.

The unretained peak time can change during programmed temperature or programmed pressure GC runs — including constant flow control of packed or capillary columns — depending on the pneumatic system and its mode of operation. Record the unretained peak at the initial column temperature and pressure to verify the instrument set-up and then maintain a complete description of the method temperature and pneumatic conditions from beginning to end.
GC operators should determine the unretained peak time and examine its shape after column installation, a carrier-gas supply change or a GC method change. This step verifies that carrier gas is flowing through the column at the desired rate. The unretained peak should have a symmetrical shape; tailing or other distortions indicate a problem with the column or its installation that operators should correct before proceeding. Record the unretained peak at faster chart speeds — or expand its area in the chromatogram on the computer screen — to see its shape better.

For single packed-column analyses, the unretained peak shape helps diagnose installation or column problems, but GC methods most often specify the column flow-rate and not the unretained peak time. The unretained peak time is difficult to relate to packed column flow-rate and pressure drop, and often it is too short for accurate measurement, so chromatographers should measure the flow at the column exit and the pressure drop across the column. The relationship between flow and pressure provides a convenient diagnostic tool. After installing a new column, record the pressure drop and measure the flow with a flowmeter at the initial and final column temperatures. The pressure drop will be higher at elevated oven temperatures — make sure that it does not exceed the upper pressure limit of the pneumatics. Then check the pressure and flow values again at regular intervals. A significant change in the pressure drop at either the lower or the upper temperature indicates changes in the column packing or a developing leak that will require attention. Any peak-shape aberrations indicate an ongoing problem with the inlet or the column packing.

For capillary columns, the unretained peak time is an essential parameter that allows chromatographers to calculate the carrier-gas velocity through the column and cross-check it with the measured pressure drop. The GC method may specify the carrier-gas velocity, the unretained peak time or both in addition to the pressure drop. The velocity in the column is lower near the beginning and higher at the exit, because of expansion of the compressible carrier gas as it moves down the column. For simplicity, most chromatographers concern themselves only with the average carrier-gas velocity along the entire column. The following formula gives the average carrier-gas linear velocity ($\bar{u}$) in centimetres per second in terms of the unretained peak time ($t_{u}$) in seconds and the column length ($L$) in centimetres.

$$\bar{u} = \frac{L}{t_{u}}$$

The carrier-gas velocity affects the column's efficiency in resolving solute peaks. If the velocity is too high, solutes don’t spend enough time in the column for an efficient separation; if the velocity is too low, solutes broaden excessively by diffusion through the mobile phase. A normal range of operation exists between these two extremes that is approximately 20–40 cm/s for helium and 30–60 cm/s for hydrogen carrier gases. The exact optimum average velocity depends on the column specifications, temperature profile and solute, in addition to the carrier gas. It is impossible to choose one velocity that yields the best efficiency for all solutes in a diverse multicomponent mixture. Furthermore, large carrier-gas velocity changes in a temperature-programmed analysis affect relative peak separation as well as peak widths and may cause adjacent peaks to coelute or even reverse their elution order. For these reasons, be sure to determine the average carrier-gas linear velocity after column installation, changing carrier-gas tanks or making any changes to the inlet or pneumatic systems.

The exact relationships between the carrier-gas pressure drop and the linear velocity for capillary columns provide a diagnostic tool analogous to the relationship of pressure and flow for packed columns. For any column at a specific temperature with a known carrier gas, the pressure drop across the column dictates the average carrier-gas velocity and the column flow-rate according to relationships too complex to discuss in this “GC Connections” column. Chromatographers can use these calculations if they have access to a computer or to a gas chromatograph with electronic pneumatic controls. Electronic pneumatic control systems for capillary columns incorporate the column pressure–velocity–flow relationships into the gas chromatograph, and GC users can take advantage of this built-in capability. For those without electronic systems, carrier-gas calculators are available on the Internet: the flow calculator at http://www.chem.agilent.com/cag/servsup/ssmain.html is a good example.

To use a program such as those or the built-in capabilities of electronic pneumatic systems, enter the column dimensions, carrier-gas identity, column outlet pressure conditions and — for an external calculator — enter the column temperature. The results from these calculations are only as good as the information that users input. With an electronic pneumatic system, errors in the input column dimensions will cause the actual operating conditions to depart considerably from the set points.

Next enter the desired average carrier-gas linear velocity in the instrument system or flow calculator. The resulting column pressure is the pressure increment above atmospheric pressure required to produce the specified linear velocity through a column with the given dimensions at the current oven temperature. An electronic system will adjust the pressure automatically. With an external calculator, set the indicated pressure on the capillary inlet system by adjusting the pressure regulator accordingly.

Then inject an unretained substance and record the chromatogram. Calculate the measured average carrier-gas linear velocity from the unretained peak time, according to Equation 1, and compare it with the nominal velocity entered into the electronic system or calculator. The two velocities should be within approximately 10% of each other. A larger discrepancy indicates a potential problem with a carrier-gas leak, an error in the column dimensions, the wrong type of carrier gas or the wrong column outlet pressure setting.

The column outlet pressure indicates to the pneumatic calculator whether the column vents to the atmosphere or to a vacuum system. Larger inner-diameter columns or short columns may require inlet pressure less than atmospheric pressure to deliver the set point velocity to a vacuum system; checking the required pressure drop before operating the column helps prevent this mistake.

Wide-bore, 0.53 mm inner diameter columns present a problem with pressure drop and linear velocity correlation because the pressure drops they require for normal operation are very low, ranging from 1.5 psig helium for a 15 m column at 30 cm/s linear velocity to 6.25 psig for a 60 m column at the same velocity. Mechanical pressure gauges will not indicate these low pressures accurately, and even electronic systems will have trouble at the low end of this range. Use a direct flow-controlled inlet system, not a split inlet, with columns shorter than 25 m in length. Calibrate the flow controller, set the desired flow-rate, and check the flow at the column exit. A column flow calculator program will compute the corresponding linear velocity, so it is possible to cross-check velocity and flow for these columns.

**Column dimensions:** Most chromatographers take the column dimensions right off the column box and assume that they are correct. In fact, this assumption is a frequent source of problems. The original box or specification sheet indicates the column dimensions correctly, but after the column is removed from the box...
there is no certainty that an operator will return it to the correct box. Subsequent users may be surprised to find that the column in the box is not what the box indicates. Most columns come with a serialized identity tag attached to the column cage for this reason. Even then, the length of a capillary column becomes shorter with each installation — sometimes by as much as 1 m — which quickly invalidates the length shown on the tag. Accurate column length is essential for several computations, including the carrier-gas linear velocity. With electronic pneumatic control systems, the column length and other column dimensions play a crucial role in the instrument system’s determination of column flow and split ratio, which have a direct impact on quantitative results.

To avoid these problems, establish an inventory list for both packed and capillary columns that correlates column serial numbers with their original dimensions and packing or coating specifications. Keep a log of column activities and dates of installation and removal from service. For capillary columns, measure and record the apparent column length before installation by counting turns and computing the length from the following formula:

\[ L = n \pi d \]  

where \( n \) is the number of column coils and \( d \) is the average coil diameter on the column cage. Subtract the total length of column removed from both ends during installation. The result is accurate enough for most applications: an error of one coil on a 15 cm (6 in.) cage is equal to approximately 0.5 m, or less than 2% of a 30 m column length. Do not, under any circumstances, remove the column from its cage, stretch it out along the laboratory floor, and count floor tiles to determine the length. Not only will this damage the column by attracting dirt on the outer coating, but it may damage other laboratory personnel as they trip over the nearly invisible tubing.

GC users can assume that the column inner diameter is very close to its nominal value, because of the precision drawing machines that produce fused-silica capillary column tubing. In general, the tubing’s inner diameter lies within ±0.4–0.5% of its nominal value. With the correct column length and carrier-gas identity, the linear velocity test described above will quickly identify a mislabelled column. If the column inner diameter is unknown, a flow calculator programme will provide an indication to within roughly 10% of the actual column diameter. Simply adjust the input column diameter until the calculated pressure or linear velocity correlates with the measured value. Then round the apparent diameter up or down to the closest diameter available from the specific column manufacturer and record that value.

The unretained peak time provides a check not only of the column linear velocity that is important for obtaining the best possible column performance but also for checking the column dimensions and the electronic pneumatic system set-up. Retained peak measurements: The next items to examine in Figure 1 are the elution times of the retained solute peaks, designated by the subscripted \( R \). All solutes that pass through a column spend the same time in the mobile phase, equal to the unretained peak time. Any additional time spent in the stationary phase is equal to the difference between the peaks’ retention times \( (t_R) \) and the unretained peak times. Chromatographers call this parameter the adjusted retention time \( (t'_R) \), which is measured in seconds and can be calculated from the following formula:

\[ t'_R = t_R - t_M \]  

The adjusted retention time is not very significant by itself, but it plays an important role in another retention measurement, the retention factor \( (k) \). The retention factor is the ratio of the adjusted retention time to the unretained peak time. In other words, it expresses peak retention in terms of multiples of the unretained peak time.

The formula for the retention factor is

\[ k = \frac{t'_R}{t_M} = \frac{t_R - t_M}{t_M} \]  

Interestingly, the retention factor doesn’t depend on the average carrier-gas linear velocity. Increasing or decreasing the velocity changes both the unretained and retained peak times in proportion. This behaviour makes the retention factor very useful for column characterization with a test mixture under isothermal conditions. GC operators can detect potential problems in their separations by periodically monitoring the retention factors of test substances.

A retention time shift could be caused by changes in the column pressure drop or by changes in the column itself, such as loss of the stationary phase. Pressure-drop changes do not affect the retention factor; however, stationary-phase changes do. By regularly determining the retention factors of test solutes, chromatographers can monitor the condition of their columns and can detect small stationary-phase losses or shifts in apparent column polarity before they significantly affect peak separation and resolution.

Extended column use at temperatures close to a column’s maximum rated operating temperature can significantly reduce the amount of stationary phase in the column. Other catastrophic events, such as oxygen leakage or chemical stress can cause a sudden loss of stationary film or a change in the phase polarity. Chromatographers can track the loss of stationary phase by monitoring retention factors of test-mixture peaks as part of routine column qualification. Stationary-phase losses cause peaks to be eluted at lower, earlier retention factors under the same temperature conditions. A peak shift towards lower retention of more than 20% will indicate a continuing problem with stationary-phase loss that could affect peak separation and resolution.

Two retained peaks: The relationship of two peaks’ retentions also plays a major role in chromatographic separations. Two peaks that are close together require more resolving power from a column than peaks that are far apart. Analysts measure the relative separation of two peaks in two similar ways: the separation factor \( (\alpha) \) and the relative retention \( (r) \). These two measurements take the same values, but their fields of application are different. The separation factor is commonly used in characterizing the separating power of a particular column under specific analytical conditions, and the use of relative retention appears most often in data-handling systems. The ratio of two retention factors defines both parameters:

\[ \alpha = k_2/k_1 \] for adjacent peaks

\[ r = k_2/k_1 \] for any two peaks

In the first equation \( k_1 \) and \( k_2 \) are the retention factors of the first and second adjacent peaks, respectively, when determining the separation factor. For relative retention, \( k_j \) defines a reference peak retention factor and \( k_i \) defines the target analyte retention factor, which can be greater or less than the reference peak retention factor. The two peaks for relative retention calculation don’t have to be adjacent.

The separation factor is always greater than or equal to 1, and it always applies to two adjacent peaks. It indicates the degree of selectivity obtained with a particular
column and set of analytical conditions. Drifting separation factors in test mixture chromatograms obtained over time can indicate a continuing problem with column polarity shifts caused by stationary-phase degradation or column contamination.

The relative retention can apply to any two peaks in a chromatogram. It is more independent of long-term changes in column length or film thickness than absolute retention times or retention factors, and it is often used in data-handling system peak identification.

Significant changes in the separation factor or relative retention indicate shifting column polarity in isothermal analyses. In the instance of temperature-programmed analyses, drifting linear velocity may also have this effect.

**Peak width:** Each peak has a characteristic width, which chromatographers usually measure at a halfway point on the peak. The peak width at half-height (\(w_h\)), given in seconds, characterizes the aggregate amount of broadening that a solute has undergone in its passage through the inlet, column and detector. In most situations, the inlet and detector contributions to peak width are negligible and the column normally produces most of the broadening.

Figure 1 shows two methods for peak-width determination. The first uses direct measurement of the peak width at half-height from the chromatogram, which only requires a ruler. The second method uses the peak area and peak height to approximate the width at half-height. A triangle drawn from the peak apex to the baseline through the data points at half-height has an area approximately equal to 94% of the entire peak area (A). The following equation relates the peak area and height to the width at half-height:

\[
w_h = 0.94 A / m_{\text{max}} \quad [6]
\]

where \(m_{\text{max}}\) is the peak height at its apex. This formula is convenient when the chromatogram is stored in a computer, because it only requires the peak area and height. Many data-handling systems include the capability to report peak widths, which operators should choose over the approximation shown in Equation 6. With a peak width at hand, a GC operator can ascertain the apparent column performance level in relation to the highest performance the column could deliver or to laboratory standards for column performance.

Chromatographers measure column performance in terms of the number of theoretical plates (N) that the column delivers or in terms of the distance or height that one theoretical plate occupies in the column. High performance columns produce more theoretical plates and correspondingly smaller plate heights. Narrower peak widths translate to higher performance; the relationship between the peak width at half-height and the measured number of theoretical plates (\(N_{\text{meas}}\)) is

\[
N_{\text{meas}} = 5.545 \left( \frac{t_R}{w_h} \right)^2 \quad [7]
\]

Narrower peak widths at longer retention times have higher plate numbers. For example, a peak that is eluted at 10 min with a width at half-height of 4 s has 125000 measured theoretical plates.

Dividing the column length by the plate number yields the height equivalent to a single theoretical plate (\(H\)), in centimetres:

\[
H = L / N \quad [8]
\]

For a 30 m column that yields the peak mentioned above, the measured plate height is 0.024 cm or 240 µm.

To gauge column performance, chromatographers compute the smallest possible plate height that a column could deliver. The minimum theoretical plate height (\(H_{\text{min}}\)) is related to the column inner diameter (\(d_c\)) and the retention factor by the following formula:

\[
H_{\text{min}} = d_c \sqrt{\frac{1 + 6k + 11k^2}{12\left(1 + k^2\right)}} \quad [9]
\]

The value of the square-root function of \(k\) in Equation 9 ranges from 0.6 at a \(k\) value of 1.0 to 0.95 at a \(k\) value of 100. In this example, if the average carrier-gas linear velocity in the 30 m column were 30 cm/s, then the unretained peak time from Equation 1 would be 100 s and the retention factor from Equation 4 would be 5.0. At a \(k\) value of 5.0, the square-root function in Equation 9 is equal to 0.84. If the column has an inner diameter of 0.025 cm (250 µm), then the minimum possible plate height is 0.021 cm or 210 µm (0.03 0.0250 5 0.021 cm).

How does this theoretical value for the plate height compare with the measured value? Chromatographers can calculate the percent utilization of theoretical efficiency (UTE%) to make this comparison:

\[
UTE\% = 100 \left( \frac{H_{\text{min}}}{H_{\text{meas}}} \right) \quad [10]
\]

In this example, UTE% is 100 \times 0.021 / 0.024, which equals 87.5%. This result means that the column delivers nearly all the possible efficiency that it could for this peak.

The above calculations do not include an adjustment for the effects of the stationary-phase film on the measured plate height; they assume that no film is present. This is untrue, of course, because the column wouldn’t retain any peaks if no phase was present, and the peaks all would be eluted at the same unretained peak time. The stationary-phase film broadens peaks as the analytes traverse the interface between it and the mobile gas phase and as they diffuse slowly through the film while inside it. The extent of this stationary-phase band broadening depends primarily upon the film thickness. Thin-film columns with coatings of 0.25 µm or less suffer only negligible losses, but thick-film columns can lose as much as 50% or more of the theoretical performance.

Rather than attempt to calculate the degree of efficiency loss caused by the stationary phase, GC users should measure the plate height or number of theoretical plates when a column is new. Make sure the new column delivers close to 100% or more of the performance, as recorded on the manufacturer’s test chromatogram under the same conditions. This level of performance may be significantly less than the theoretical maximum, but it reflects the effect of the stationary film on the plate height. If a new column fails to yield performance similar to its original test chromatogram, then carefully check the column installation and the linear velocity. For an old column, chromatographers should expect some performance degradation over time. Replacement is mandated when the column no longer delivers a satisfactory chromatogram for the test mixture or analytical standard, fails to deliver a minimum plate number, or tests to a larger plate height than tolerated by the test procedure. Any of these column qualification criteria are equally valid; operators should choose one and apply it regularly.

**Conclusion**

GC users can perform many basic chromatographic calculations based on a test chromatogram, the pressure drop across the column, its temperature and the column dimensions. Making these basic measurements and calculations can provide chromatographers with enhanced quality assurance for their analytical results. Testing following installation and at regular intervals will provide a record of column performance over time that analysts can use to gauge when to replace a column, as well as to identify problems before they
become serious and significantly affect analytical results.

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