Column length also plays a role in the separation resolution. As column length changes, the efficiency (N) changes in direct proportion to the ratio of the column length (27). Resolution, as indicated in the resolution equation (vide supra), changes as a function of the square root of the change in N, and an estimate of the change in resolution as a function of column length can be approximated with the equation

$$R_{s2} = R_{s1} \cdot \left(\frac{L_2}{L_1}\right)^{1/2}$$

where R_{s1} is the resolution obtained from column 1 and R_{s2} is the estimated resolution with column 2.

Similarly the run time (RT) and column back pressure (P) will also change in direct proportion to a change in the column length by

$$RT_2 = RT_1 \cdot \frac{L_2}{L_1} \qquad P_2 = P_1 \cdot \frac{L_2}{L_1}$$

where RT_1 is the run time for column 1, RT_2 the run time for column 2, P_1 the pressure for column 1, and P_2 the pressure for column 2.

While most analytical columns are standardized to a 4.6 mm id, their lengths vary; they are available in lengths of 5 cm, 15 cm, and 25 cm, whereas the original Waters μBondapak® C18 column measures 30 cm × 3.9 mm id. A good selection of columns illustrating type and sizes can be found in most HPLC vendors' supply catalogs.

Claessens et al. (28) have reported on an extensive study on the effect of buffers on silica-based column stability in reversed-phase HPLC. As the analytical column has a silica-based backbone, it is not stable in alkaline pH. The authors reported that silica-based bonded phase packings variably degrade with buffers as a function of the type of anion, cation, pH, buffer type, and temperature.

10.9. Role of Temperature

While temperature is a variable that can affect selectivity, α, its effect is relatively small. Also, the k' generally decreases with an increase in temperature for neutral compounds but less dramatically for partially ionized analytes. Still, it may have some effect when there is a significant difference in shape and size between samples. Overall, it is better to use solvent strength to control selectivity than to use temperature; its effect is much more dramatic. Snyder et al. (29) reported that an increase of 1°C will decrease the k' by 1 to 2%, and both ionic and neutral samples are reported to show significant changes in α with temperature changes. Because of possible temperature fluctuations during method development and validation, it is recommended that the column be thermostated to control the temperature.

10.10. Role of Flow Rate

Flow rate, more for isocratic than gradient separation, can sometimes be useful and readily utilized to increase the resolution, although its effect is very modest. The slower flow rate will also decrease the column back pressure. The disadvantage is that when flow rate is decreased, to increase the resolution slightly, there is a corresponding increase in the run time.