Chromatography Solutions

Knowledge note #0003

How to Calculate Resolution

INTRODUCTION

The primary aim of a chromatographic separation is to achieve adequate separation of analyte peaks. Baseline separation enables the chromatographer to quantify accurately the area or height of a peak. Resolution is a quantitative measure of how well two eluted peaks are separated and is easily measured from the peak retention times and peak widths.

CALCULATION

The resolution (R_S) of two chromatographic peaks can be defined using either the peak width measured at the base or the peak width measured at half height, as shown in equations 1 and 2 respectively.

$$R_S = \frac{2(t_{R2} - t_{R1})}{(w_{b1} + w_{b2})} \quad (1)$$

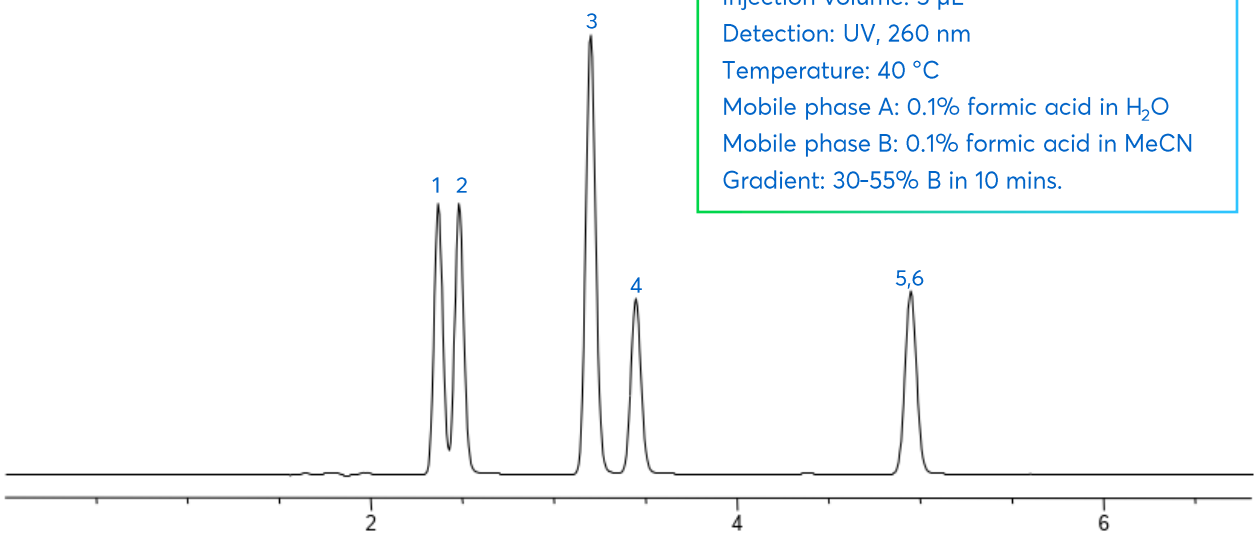
$$R_S = \frac{1.176(t_{R2} - t_{R1})}{(w_{0.5,1} + w_{0.5,2})} \quad (2)$$

Where w_b and $w_{0.5}$ are the peak widths measured at the base and half height and t_R is the retention time.

The United States Pharmacopoeia (USP) specifies the use of baseline peak width, whereas the British and European pharmacopoeias specify peak width at half height. It is important to verify, with any pharmacopoeia or regulated method, which equation is required.

It is generally accepted that an R_S value of greater than 1.5 indicates baseline resolution. However, to account for the possibility of peak tailing, a minimum resolution of 1.7 is often desirable. Examples of peaks that are fully resolved (peaks 3 & 4), partially resolved (peaks 1 & 2) and completely co-eluting (peaks 5 & 6) are shown in Figure 1.

Column : Avantor® ACE® 3 C18, 150 x 4.6 mm
Flow rate: 1 mL/min
Injection volume: 5 µL
Detection: UV, 260 nm
Temperature: 40 °C
Mobile phase A: 0.1% formic acid in H_2O
Mobile phase B: 0.1% formic acid in MeCN
Gradient: 30-55% B in 10 mins.



Peak	Analyte	t_R	W_b	R_S
1	4-Hydroxybenzoic Acid	2.366	0.0902	1.24
2	Vanillic Acid	2.48	0.0938	7.30
3	4-Hydroxybenzaldehyde	3.198	0.1029	2.36
4	Vanillin	3.444	0.1055	13.44
5	Guaiacol	4.946	0.1180	0.00
6	Ethyl vanillin	4.946	0.1180	0.00

Figure 1: Separation of vanillin compounds demonstrating fully resolved, partially resolved and co-eluting peak pairs.