

# Performance Testing of Packed Columns

## Guidelines for Performance Testing of Packed Columns

To check the quality of the column packing, the theoretical number of plates and peak asymmetry must be established. This is achieved by quantifying the geometry of an isocratic peak, which can be produced by injection of a marker detectable by spectrophotometry, pH or conductivity.

For hydrophobic ligands a solution such as 2M NaCl should be used. NaCl will not interact with the ligand chemistry in such a way as to cause an anomalous result. Otherwise a solution of 2% (v/v) acetone may be used. The injection volume should be 0.1 – 0.5 % of the column volume.

Number of plates can be calculated using the following equation:

$$N = 5.54(t_r / W_{1/2})^2$$

where:

- $N$  = number of theoretical plates
- $t_r$  = retention time of marker molecule
- $W_{1/2}$  = width of peak at half its maximum deflection

Refer to the graph overleaf for illustration of these parameters. For the calculation to work, the time and width values must both be reported as the same units of measure: geometric lengths observed on the chromatogram, or the time observed for both events to occur.

To allow comparison of different columns with the same media, the number of theoretical plates *per metre* must be calculated, as follows:

$$N_L = N / L$$

where:

- $N_L$  = number of theoretical plates per metre
- $N$  = number of theoretical plates
- $L$  = height of packed bed in metres

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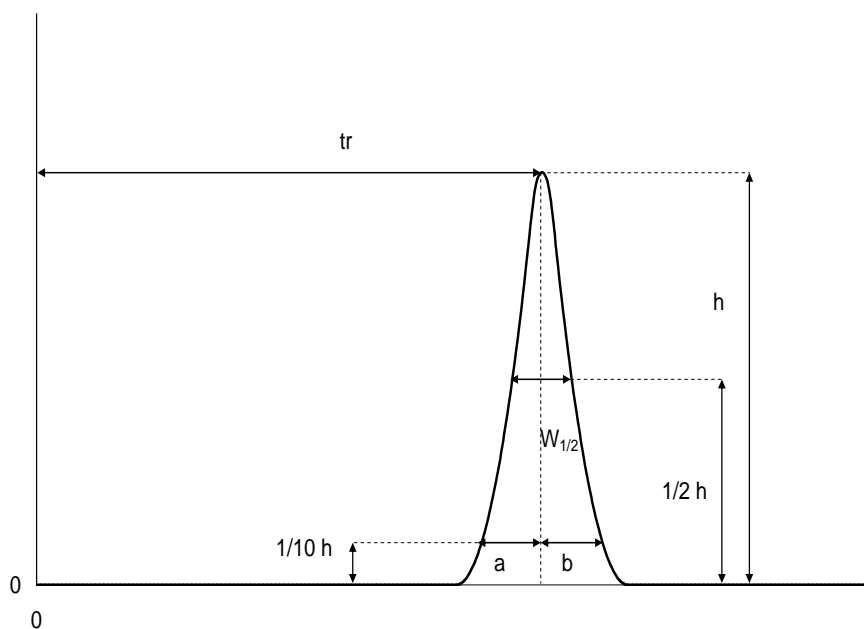
Asymmetry can be calculated as follows:

$$A_s = b / a$$

where:

- $a$  = the distance from the leading edge of the peak to the mid-point of the peak
- $b$  = the distance from the mid-point of the peak to trailing edge.

$A_s$  is measured using data from 5 - 10% of the maximum peak height.



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