Performance Measurement and Column Efficiency

To determine chromatographic efficiency of a packing chromatography column it is usual practice to inject a small analytical sample onto the column and monitor the response at the outlet.
Typical solutions used are acetone in water or a 1M NaCl solution. If acetone solution is used then the UV absorbance is measured and plotted. If NaCl solution is use then conductivity is measured and plotted.
The plot obtained is then analyzed to calculate the following terms of efficiency.

Chromatographic efficiency is expressed as HETP (height equivalent to theoretical plate) .This is calculated direct from the UV plot as shown in the diagram below.
Calculation of HETP,

HETP =

Where L is the column height in mm
Where N is the number of theoretical plates

Calculation of theoretical plates N

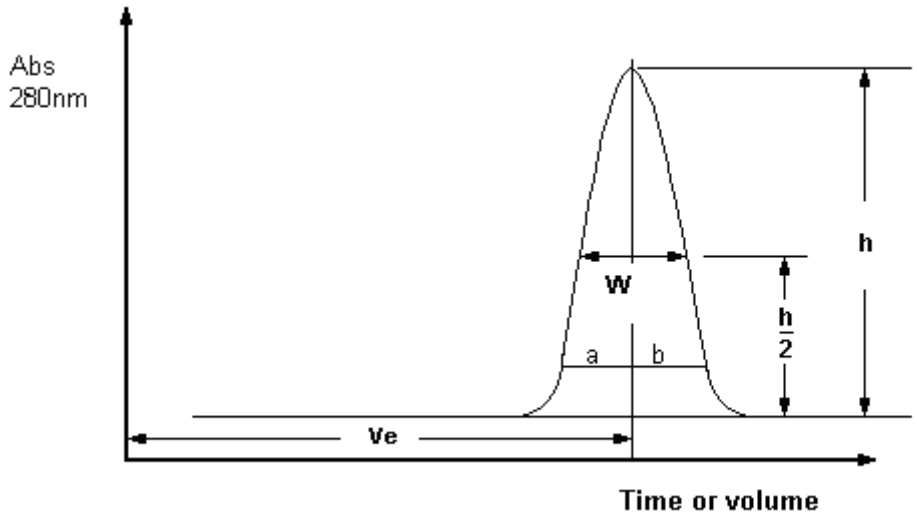

Figure A1 Description of Column Efficiency Calculations

Asymmetry is measured at 10% of the peak height and is calculated according to the equation shown below, where a and b relate to the width of the pea section shown in Figure 1.

Two further calculations can be performed to describe column efficiency.

Reduced plate height takes into account the particle size of the media and enables comparison between different media types. A low value of RPH denotes good column efficiency; a RPH of less than 6 is expected.

Where P_d is the mean particle diameter.

Dilution factor includes the initial sample volume in the calculation. This allows comparison between different peak shapes. The dilution factor is given by the following equation.

Where the initial sample volume and the values of a and b are all expressed in the same volume units.

Comparison of results should be undertaken with caution.
Different media manufacturers often express performance in terms of the definitions above. Caution must be taken when interpreting performance results. It is advised to check not only the media type used but also the flowrate, column diameter and bed height. Often results are given for tests performed in laboratory scale columns.

Performance Results From Evolve™ Columns

Performance tests have been performed on the Evolve™ range of columns using ProMetic chromatography media. This media is an agarose based material with a particle size of 90micron.

Accepted performance results for this media as quoted by ProMetic is expressed in number of plates per metre and asymmetry.
Plates per metre should be greater than 2,500 and asymmetry values between 0.8- 1.4

Bed heights of 15-20cm were packed at ambient temperature using filtered deionised water as the packing buffer. The packed beds were quantified using a 2% acetone solution and the sample size was equivalent to 0.5cm band width.
The table below shows results when a flowrate of 100cm/hr was used.

Column	**N, Number of plates**	**Asymmetry**
350	3913	1.11
250	5352	1.12
200	4611	1.04
100	4308	1.13

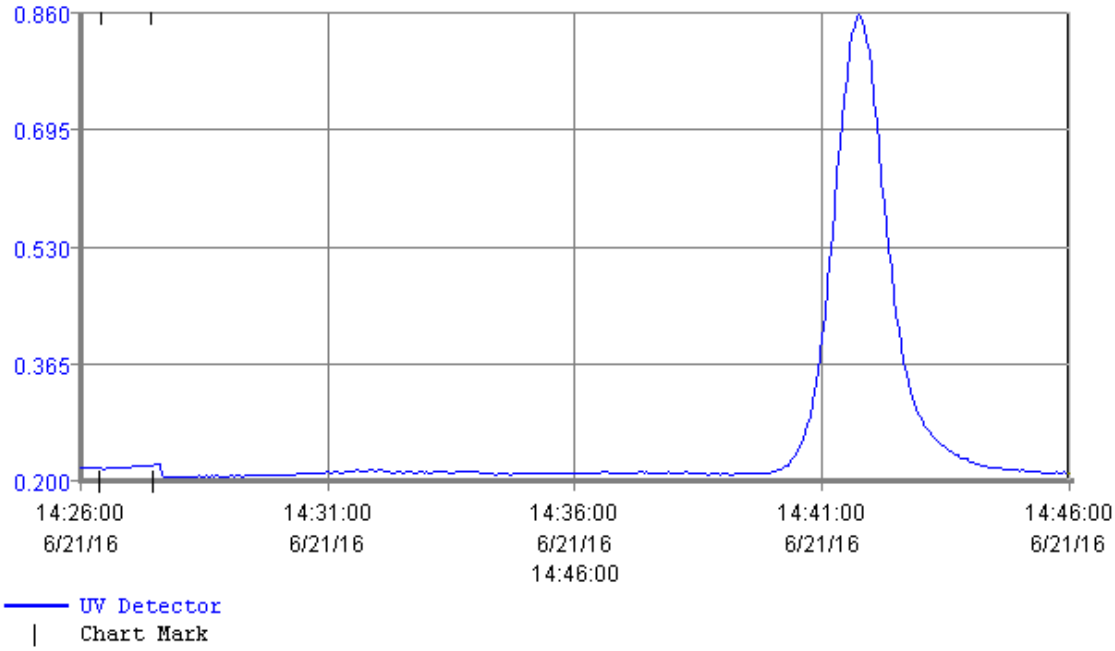

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Figure A2 Example of typical chromatogram. Evolve™ 350mm column. 2% Acetone sample, flowrate 100cm/hr, bed height 19cm.