Waters
Lab Highlights
An Internal Communication
of Applications and Techniques
LAH 0251 5/85
AN/OT/ED/OT/OT

Determining Column Efficiency Using the Model 840 Data System

Measuring column efficiency (or system bandspreading) is a simple process, but it requires care in drawing baselines, constructing perpendiculars, and making precise measurements with a ruler. The model 840 Data System provides a way to perform these measurements using only the 840 and a pocket calculator.

1. Run a column efficiency test as described in LAH 0129. Use a sampling rate of 10 points/second, and run a test chromatogram and measure the peak width at baseline (for a Nova-Pak™ C18 column, use 20 seconds).
2. Reprocess the data using SCANNER. When asked for a method name, use the default method, or the method used for the run.
3. Bring the test peak on the screen, and use the arrow keys to place the cursor on the baseline either before or after the peak.
4. Record the millivolt reading in the upper left hand corner of the screen. (this is the detector output at baseline, V_b).
5. Using the arrow keys, place the cursor at the apex of the peak, and record the millivolt reading (the detector output at the peak maximum, V_{max}).
6. Calculate the net peak height as $H = V_{max} - V_b$.
7. Calculate the height at 4.4% as $H_{4.4} = (H \times 0.044) + V_b$.
8. Using the arrow keys, place the cursor near the beginning of the peak. Move the cursor until the millivolt reading is as close to $H_{4.4}$ as you can get it*, and press "SELECT". Move the cursor to the end of the peak, and again adjust the position until you are close to $H_{4.4}$, and press "FIND". The peak will be integrated, and a line of data will appear above the peak. Press "PRINT SCREEN", or record the retention time T_R (the first figure in the line of data), the start time T_S , and the end time T_E.
9. Calculate the column efficiency as $N = 25 \times [T_R /(T_E - T_S)]^2$. If you are measuring system bandspreading, just convert T_S and T_E into volume in microliters: **VOL = T X F X 1000**, where F = flow rate in ml/min.

*Normally you will not be able to get the reading to match $H_{4.4}$ exactly, but the error in the plate count shouldn't be more than 5%, which is close enough for diagnosing most column problems. If you want a more accurate value, you can use interpolation to calculate T_S & T_E.

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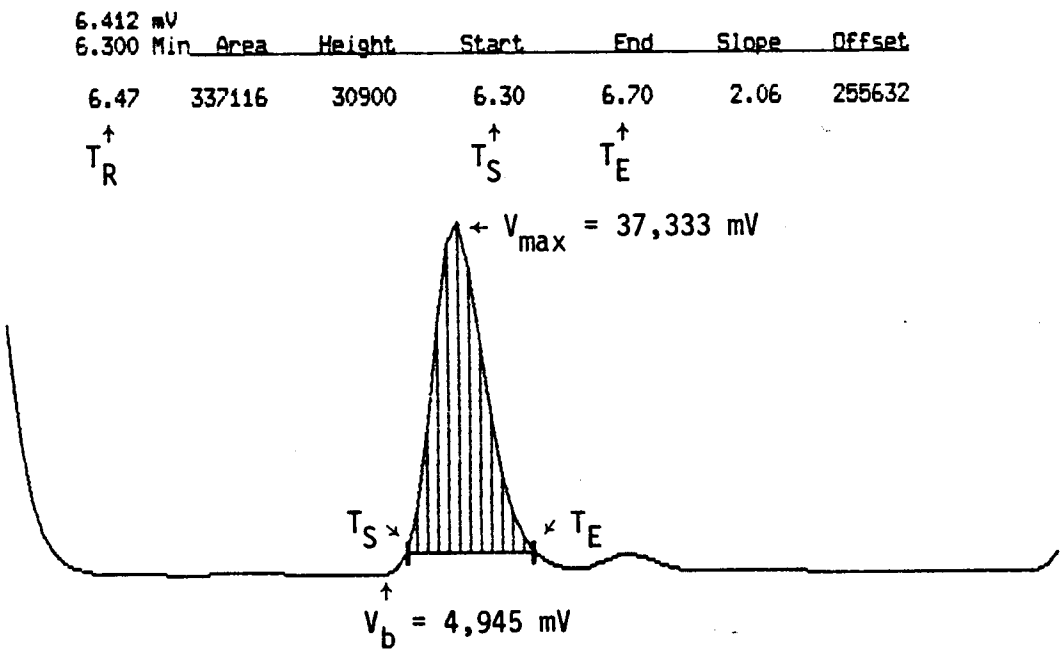

In the example shown above, the efficiency was determined as follows:

Determination of net peak height

$V_b = 4.945$ $V_{max} = 37.333$

$H = 37.333 - 4.945 = \underline{32.388\ mV}$

Calculation of height at 4.4%

$H_{4.4} = (32.388 \times 0.044) + 4.945 = \underline{6.370\ mV}$

Determination of retention time, start time and end time (see figure)

$T_R = 6.47min$ $T_S = 6.30min$ $T_E = 6.70min$

Calculation of efficiency

$N = 25 \times [6.47/(6.70 - 7.30)]2 = \underline{6541}.$