Agilent Technologies

Evaluating System Suitability
CE, GC, LC and A/D ChemStation
Revisions: A.03.0x- A.08.0x

This document is believed to be accurate and up-to-date. However, Agilent Technologies, Inc. cannot assume responsibility for the use of this material. The information contained herein is intended for use by informed individuals who can and must determine its fitness for their purpose.

Windows® and Windows NT® are registered trademarks of Microsoft Corp

Evaluating System Suitability

Evaluating the performance of both the analytical instrument before it is used for sample analysis and the analytical method before it is used routinely is good analytical practice. It is also a good idea to check the performance of analysis systems before, and during, routine analysis. The HP ChemStation software provides the tools to do these three types of tests automatically. An instrument test can include the detector sensitivity, the precision of peak retention times and the precision of peak areas. A method test can include precision of retention times and amounts, the selectivity, and the robustness of the method to day-to-day variance in operation. A system test can include precision of amounts, resolution between two specific peaks and peak tailing.

Laboratories which have to comply with:

- Good Laboratory Practice regulations (GLP),
- Good Manufacturing Practice regulations (GMP) and Current Good Manufacturing Practice regulations (cGMP), and
- Good Automated Laboratory Practice (GALP)

are advised to perform these tests and to document the results thoroughly. Laboratories which are part of a quality control system, for example, to comply with ISO9000 certification, will have to demonstrate the proper performance of their instruments.

The HP ChemStation collates results from several runs and evaluates them statistically in the sequence summary report.

The tests are documented in a format which is generally accepted by regulatory authorities and independent auditors. Statistics include:

- peak retention time,
- peak area,
- amount,
- peak height,
- peak width at half height,
- peak symmetry,

- peak tailing,
- capacity factor (k´),
- plate numbers,
- resolution between peaks,
- selectivity relative to preceding peak,
- skew, and
- excess

The mean value, the standard deviation, the relative standard deviation and the confidence interval are calculated. You can set limits for either standard deviation, the relative standard deviation or the confidence interval for each of these parameters. Should the values exceed your limits, the report is flagged to draw your attention to them.

The quality of the analytical data can be supported by keeping records of the actual conditions at the time the measurements were made. The HP ChemStation's logbook records instrument conditions before and after a run. This information is stored with the data and reported with sample data. Instrument performance curves are recorded during the entire analysis as signals, and stored in the data file. If supported by the instrument these records, overlaid on the chromatogram/electropherogram, can be recalled on demand, for example, during an audit.

Baseline noise and drift can be measured automatically. A minimum detectable level can be calculated from peak height data for each calibrated compound in the method.

Finally, instrument configuration, instrument serial numbers, column identification, and your own comments can be included in each report printed.

Extended performance results are calculated only for compounds calibrated for in the method, ensuring characterization by retention times and compound names.

A typical system performance test report contains the following performance results:

- instrument details,
- column/capillary details,
- analytical method,
- sample information,
- acquisition information,
- signal description and baseline noise determination, and
- signal labeled with either retention/migration times, or compound names.

In addition, the following information is generated for each calibrated compound in the chromatogram/electropherogram:

- retention/migration time,
- k´,
- symmetry,
- peak width,
- plate number,
- resolution,
- signal-to-noise ratio, and
- compound name.

Noise Determination

Noise can be determined from the data point values from a selected time range of a signal. Noise is treated in three different ways:

1 as six times the standard deviation (sd) of the linear regression of the drift,

2 as peak-to-peak (drift corrected), and

3 as determined by the ASTM method (ASTM E 685-93).

Figure 41 **Noise as Six Times the Standard Deviation**

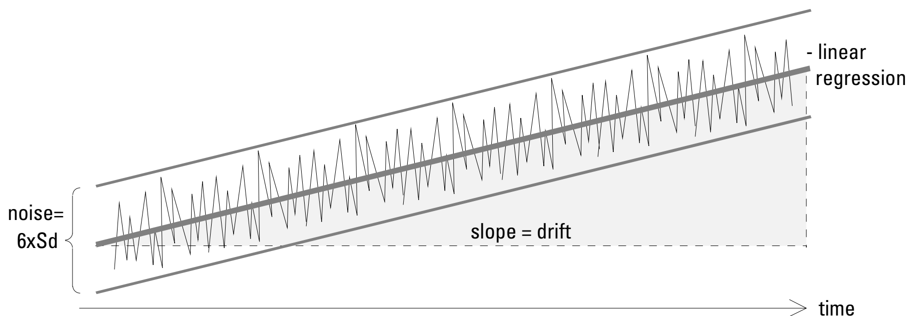
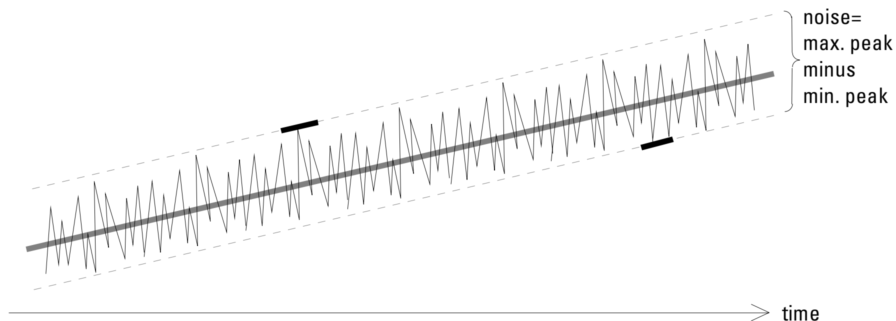
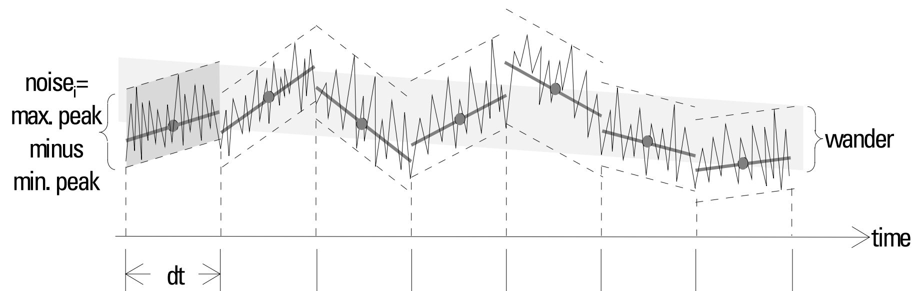


Figure 42 **Noise as Maximum Peak to Minimum Peak (Distance)**



Drift is given as the slope of the linear regression, see Figure 41, and wander is determined as the peak-to-peak noise of the mid-data values in the ASTM noise cycles, see Figure 43.

Figure 43 **Noise Determined by the ASTM Method**



ASTM noise determination (ASTM E 685-93) is based on the standard practice for testing variable-wavelength photometric detectors used in liquid chromatography, as defined by the American Society for Testing and Materials. Based on the size of the time range, three different types of noise can be distinguished. Noise determination is based on peak-to-peak measurement within defined time ranges.

1 Long-term noise—the maximum amplitude for all random variations of the detector signal of frequencies between 6 and 60 cycles per hour. Long-term noise is determined when the selected time range exceeds one hour. The time range for each cycle (dt) is set to 10 minutes which will give at least six cycles within the selected time range.

2 Short-term noise—the maximum amplitude for all random variations of the detector signal of a frequency greater than one cycle per minute. Short-term noise is determined for a selected time range between 10 and 60 minutes. The time range for each cycle(dt) is set to one minute which will give at least 10 cycles within the selected time range.

3 Very-short-term noise (not part of ASTM E 685-93)—this term is introduced to describe the maximum amplitude for all random variations of the detector signal of a frequency greater than one cycle per 0.1 minute. Very-short-term noise is determined for a selected time range between 1 and 10 minutes. The time range for each cycle (dt) is set to 0.1 minute which will give at least 10 cycles within the selected time range.

An ASTM noise determination is not done if the selected time range is below one minute. Depending on the range, if the selected time range is greater than, or equal to one minute, noise is determined using one of the ASTM methods previously described. At least seven data points per cycle are used in the calculation. The cycles in the automated noise determination are overlapped by 10 %.

Calculation of Peak Symmetry

The HP ChemStation does not determine the asymmetry ratio of a peak, usually done by comparing the peak half-widths at 10% of the peak height, or 5% as recommended by the FDA.

Peak symmetry is calculated as a pseudomoment by the integrator using the following moment equations:

$$m_1 = a_1\left(t_2 + \frac{a_1}{1.5H_f}\right)$$

$$m_2 = \frac{a_2^2}{0.5H_f + 1.5H}$$

$$m_3 = \frac{a_3^2}{0.5H_r + 1.5H}$$

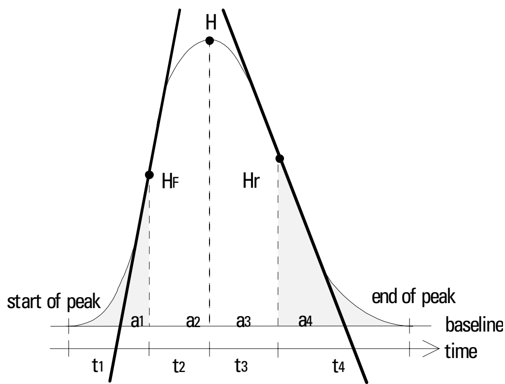
$$m_4 = a_4\left(t_3 + \frac{a_4}{1.5H_r}\right)$$

$$\text{Peak symmetry} = \sqrt{\frac{m_1 + m_2}{m_3 + m_4}}$$

If no inflection points are found, or only one inflection point is reported, then the peak symmetry is calculated as follows:

$$\text{Peak symmetry} = \frac{a_1 + a_2}{a_3 + a_4}$$

Figure 44 **Calculation of the Peak Symmetry Factor**



where:

a_i = area of slice

t_i = time of slice

H_f = height of front inflection point

H_r = height of rear inflection point

H = height at apex

System Suitability Formulae and Calculations

The HP ChemStation uses the following formulae to obtain the results for the various System Suitability tests. The results are reported using the Performance, Performance + Noise and Performance + Extended report styles.

When ASTM or USP is specified for a given definition, then the definition conforms to those given in the corresponding reference. However, the symbols used here may not be the same as those used in the reference.

The two references used in this context are:

1 *ASTM: Section E 682 – 93, Annual Book of ASTM Standards, Vol.14.01*

2 *USP: The United States Pharmacopeia, XX. Revision, pp. 943 - 946*

General Definitions

Void Volume

$$V = d^2 \pi l (f/4)$$

where:

d = diameter of column [cm]

π = constant, ratio of circumference to diameter of a circle

l = length of column [cm]

f = fraction of column volume that is not taken up by stationary phase but available for mobile phase; default value for $f = 0.68$ (for Hypersil)

Retention Time of Unretained Compound t (m) [min]

(Also referred to as dead time or void time)

$$T_m = V/F$$

where:

F = flow rate of LC [ml/min]

Performance Test Definitions

Statistical Moments

$$M0 = d_t \cdot X$$

$$M1 = t_0 + d_t \cdot \frac{X}{Y}$$

$$M2 = \frac{d_t^2}{X} \cdot \sum_{i=1}^{N} \left(\left(i - 1 - \frac{Y}{X} \right)^2 \cdot A_i \right)$$

$$M3 = \frac{d_t^3}{X} \cdot \sum_{i=1}^{N} \left(\left(i - 1 - \frac{Y}{X} \right)^3 \cdot A_i \right)$$

$$M4 = \frac{d_t^4}{X} \cdot \sum_{i=1}^{N} \left(\left(i - 1 - \frac{Y}{X} \right)^4 \cdot A_i \right)$$

where:

N = number of area slices

A_i = value of area slice indexed by i

$\sum_{i=1}^{N}$ = sum of starting index 1 to final index N for discrete observations

d_t = time interval between adjacent area slices

t_0 = time of first area slice

$$X = \sum_{i=1}^{N} (A_i)$$

$$Y = \sum_{i=1}^{N} ((i - 1)A_i)$$

Statistical Moments, Skew and Excess

Statistical moments are calculated as an alternative to describe asymmetric peak shapes. There is a infinite number of peak moments, but only the first five are used in connection with chromatographic peaks.
These are called **0th Moment**, **1st Moment**, … **4th Moment**.

The **0th Moment** represents the peak area.

The **1st Moment** is the mean retention time, or retention time measured at the center of gravity of the peak. It is different from the chromatographic retention time measured at peak maximum unless the peak is symmetrical.

The **2nd Moment** is the peak variance which is a measure of lateral spreading. It is the sum of the variance contributed by different parts of the instrument system.

The **3rd Moment** describes the vertical symmetry or skew. It is a measure of the departure of the peak shape from the Gaussian standard. The skew given additionally in the Performance & Extended report is its dimensionless form. A symmetrically peak has a skew of zero. Tailing peaks have positive skew and their 1. Moment is greater than the retention time. Fronting peaks have negative skew and their 1. Moment is less than the retention time.

The **4th Moment** or excess is a measure of the compression or stretching of the peak along a vertical axis, and how this compares to a Gaussian standard for which the 4. Moment is zero. It can be visualized by moving in or pulling apart the sides of a Gaussian peak while maintaining constant area. If the peak is compressed or squashed down in comparison, its excess is negative. If it is taller, its excess is positive. Also the excess is given in the Performance & Extended report in its dimensionless form.

True Peak Width W_x [min], [s]

W_x = width of peak at a height x % of total height

Special cases:

W_B = base width, 4 sigma, obtained by intersecting tangents through the inflection points with the baseline

$W_{4.4}$ = width at 4.4% of height, 5 sigma width

$W_{5.0}$ = width at 5% of height, used for USP tailing factor

$W_{50.0}$ = width at 50% of height, half width, 2.35 sigma

W_S = width derived from statistical moments = $\sqrt{(M2)}$

Capacity Factor (USP), Capacity Ratio (ASTM) k′

$$k' = \frac{T_R - T_0}{T_0}$$

where:
T_R = retention time of peak [min]
T_0 = void time [min]

USP Tailing Factor (USP) t

$$t = \frac{W_{5.0}}{t_w \cdot 2}$$

where:
t_w = distance between peak front and T_R at 5 % of peak height; units are the same as used for $W_{5.0}$, for example [min]

Number of Theoretical Plates per Column (USP, ASTM) n

Tangent method (USP, ASTM):

$$n = 16\left(\frac{T_R}{W_B}\right)^2$$

Half-width method (ASTM):

$$n = 5.54\left(\frac{T_R}{W_{50}}\right)^2$$

5 Sigma method:

$$n = 25\left(\frac{T_R}{W_{4.4}}\right)^2$$

Variance method:

$$n = \frac{M1^2}{M2}$$

Number of Theoretical Plates per Meter N [1/m]

$N = 100\frac{n}{l}$ where: l = length of column

Relative Retention (USP, ASTM), Selectivity Alpha

(Pertaining to peaks a and b, T_R of peak a < T_R of peak b)

$$alpha = \frac{k'_{(b)}}{k'_{(a)}}, \ alpha \geq 1$$

where:

$k'_{(x)}$ = capacity factor for peak x

Resolution (USP, ASTM) R

(Pertaining to peaks a and b, T_R of peak a < T_R of peak b)

Tangent method (USP, ASTM):

$$R = \frac{2(T_{R(b)} - T_{R(a)})}{W_{B(b)} + W_{B(a)}}$$

5 Sigma method:

$$R = \frac{2.5(T_{R(b)} - T_{R(a)})}{W_{4.4(b)} + W_{4.4(a)}}$$

Half-width method:

$$R = \frac{(2.35/2)(T_{R(b)} - T_{R(a)})}{W_{50(b)} + W_{50(a)}}$$

Statistical method:

$$R = \frac{(M_{1(b)} - M_{1(a)})}{W_{S(b)} + W_{S(a)}}$$

where:

$M_{1(x)}$=mean retention time for peak x (1st Statistical Moment)

$W_{B(x)}$= base width for peak x

$W_{4.4(x)}$= width at 4.4% height for peak x

$W_{50(x)}$= width at 50% height for peak x

$W_{S(x)}$= width derived from variance (M2) of peak x

Definitions for Reproducibility

Mean M

$$M = \frac{\sum_{i=1}^{N} X_i}{N}$$

where:

N = number of discrete observations

X_i = value of discrete observations indexed by i

Standard Deviation

$$S = \sqrt{\frac{\sum_{i=1}^{N} (X_i - M)^2}{N - 1}}$$

Relative Standard Deviation RSD[%] (USP)

$$RSD = 100\frac{S}{M}$$

Standard Deviation of the Mean

$$S_M = \frac{S}{\sqrt{n}}$$

Confidence Interval

$$CI = S_M \cdot t_{95;(n-1)}$$

where:

t 95;(n-1) = percentage point of the t distribution table, 95 % means a risk

probability of 0.025 (the t distribution is also known as the student distribution)

Regression Analysis

Let

N = number of discrete observations

X_i = independent variable, i^{th} observation

Y_i = dependent variable, i^{th} observation

Linear function: $y_{(X)} = a + bX$

Coefficients:

$$a = \frac{1}{\Delta_X}\left(\sum_{i=1}^{N} X_i^2 \cdot \sum_{i=1}^{N} Y_i^{\,2} - \left(\sum_{i=1}^{N} X_i \cdot \sum_{i=1}^{N} X_i Y_i\right)\right)$$

$$b = \frac{1}{\Delta_X}\left(N \cdot \sum_{N}^{i=1} X_i Y_i - \left(\sum X_i \cdot \sum Y_i\right)\right)$$

where:

$$\Delta_X = N \cdot \sum_{i=1}^{N} X_i^2 - \left(\sum_{i=1}^{N} X_i\right)^2$$

Regression Coefficient

$$r^2 = \frac{\left(N \cdot \sum_{i=1}^{N} X_i Y_i - \left(\sum_{i=1}^{N} X_i \cdot \sum_{i=1}^{N} Y_i\right)\right)^2}{\Delta_x \cdot \Delta_y}$$

where:

$$\Delta_Y = N \times \sum_{i=1}^{N} Y_i^2 - \left(\sum_{i=1}^{N} Y_i\right)^2$$

Standard Deviation (S)

$$S = \sqrt{\frac{\sum_{i=1}^{N} (Y_i - a - bX_i)^2}{N-2}}$$