

A Method for Determining Column Performance Independent of Instrument Variance

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Abstract

HPLC columns are tested by the manufacturer to ensure that they have been packed efficiently. This is done using highly optimised LC systems. Peak broadening, or "dispersion", not only occurs in the column, but also in the dead volume in the injector, detector and connecting tubing and can have a significant effect on the measured efficiency. Instruments that are poorly configured may result in differences in 50% or more of the theoretical plates demonstrated by a manufacturer, with this being more significant on shorter columns or columns packed with smaller particles. We will demonstrate an approach to measuring instrument variance so that this can be factored into a comparison with data provided on a column test certificate.

Introduction

Efficiency is used by chromatographers to describe the performance of a column and is calculated as follows:

Equation 1: Efficiency at half height

$$N = \frac{t_R^2}{\sigma} = 5.54 \left(\frac{t_R}{W_{0.5}} \right)^2$$

Variance, σ^2 (v) is a function of peak width (w) and there are several methods by which peak width can be calculated. The most common methods of measuring this are at (i) half peak height, (ii) the tangent and (iii) the baseline.

On Thermo Fisher Scientific test certificates efficiency values are calculated by measuring the peak widths at half height.

Efficiency is not only dependent on method, analytes and column, but the instrument also contains dead volume, which contributes to broader peaks and lower efficiency values (equation 2). This extra-column volume is present in the injector, connective tubing and the detector. Repeating a method, using the same column, on a different instrument may generate different efficiency values. When replicating a manufacturer's test method, it may be important to take account of differences in the variance contribution of the different instruments.

Equation 2: Contribution of variances from different parts of a chromatographic system

$$\sigma_{total}^2 = \sigma_{injector}^2 + \sigma_{pre-column}^2 + \sigma_{column}^2 + \sigma_{post-column}^2 + \sigma_{detector}^2$$

The contribution by the instrument to the reduced efficiency values can be measured as variance. We have evaluated the variance on several instruments and have demonstrated a method which would allow a chromatographer to evaluate their own instrument's variance, and then use this to account for differences that they might observe when comparing efficiency values on a test certificate with values they themselves have produced.

Materials & Methods

HPLC method:

Columns: Thermo Scientific Hypersil GOLD 3µm 4.6mm ID with varying column length 30, 50, 100 and 150mm
Mobile Phase: Water : Acetonitrile (50:50 v/v)
Flow Rate: 1.0mL/min
Column Temperature: 40°C
Detection: 254nm
Injection Volume: 5µL

HPLC Instrumentation:

Thermo Scientific Surveyor (San Jose, CA),
Agilent Technologies HP 1100 Series HPLC System

Test certificate sample: o-xylene; 0.7% v/v in mobile phase.

Results & Discussion

Comparison of Variance on Different HPLC Instruments

Four Hypersil GOLD™ columns with different column lengths were tested on four different HPLC systems. The columns were specifically selected to ensure that these were manufactured on the same packing pump and had similar column efficiency values (plates/metre). A Surveyor™ instrument, with narrow bore PEEK connective tubing of the minimum possible length, and a 1cm²µL flow cell was our benchmark for this exercise. A standard Surveyor and an Agilent™ 1100 were also used in this comparison. Our aim was not to compare instruments, but to simply show how a range of instruments and their installation can have an effect on the efficiency of the whole system.

Peak efficiencies were used to estimate column variance, and when the variance was extrapolated to a zero column length, this was taken an estimate of the instrument variance (figure 2).

Figure 2. Plot of measured column variance against column length, the intercept represents instrumental variance

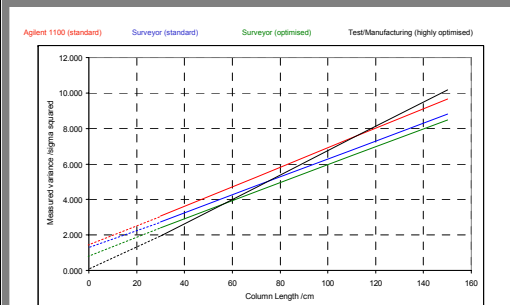


Table 1: Instrumentation and corresponding variance as calculated by the y-intercept in figure 2.

System	Instrument variance (σ²)
Agilent	1.4
Surveyor (standard)	1.2
Surveyor (optimised)	0.9
Test / Manufacturing (highly optimised)	0.1

Instrument variance values for the four instruments tested are presented (table 1). The optimized Surveyor demonstrated reduced dispersion over the non-optimized Surveyor, with respective variance values of 0.9σ² and 1.2σ².

Instruments used in producing test certificates are specially customized to minimize peak broadening so that problems with the packing of a column can be easily seen. These instruments have an estimated variance of 0.1σ², which is far less than that seen on commercially available instruments.

Method for Determining Instrument Variance

Using the variance for the instrument used to produce the column test certificates (0.1σ²), it is now possible to estimate the variance of any other instrument using equation 4.

Instrument variance has a more significant effect on the chromatography when the particle size and column length are kept to a minimum, with 4.6mm diameter columns being found to provide good packing efficiency. We used 3µm 4.6 x 50mm for our subsequent instrument evaluations.

Calculating the Expected Column Efficiency Based on Known Instrument Variance Values

Once the variance is known for the manufacturer's instrument, and that of the evaluation instrument, then it is then possible to translate the efficiency value on the test certificate to a value that we would expect to see on the evaluation instrument using equation 5.

Instrument and Column Evaluation Calculator

We have constructed an "Instrument and Column Evaluation Calculator" to help account for variance differences in the manufacturer's instrument and the instrument used by a customer, when reproducing a test certificate.

The calculator uses the difference in the efficiency on the test certificate of column and that obtained on the evaluation system in order to provide a value for the amount of dispersion on that system. If the dispersion is greater than 1.5 σ² then there may be opportunities to reduce the instrument volume by using shorter, narrow bore tubing

Having established the variance contribution of the evaluation instrument, the calculator takes the efficiency value from the test certificate of a different column. The calculator will then calculate the efficiency value that we would be expected to see for this column using the evaluation instrument. If the column is then tested using the same method stated on the test certificate and the actual efficiency is much less than the expected efficiency then there may be damage to the column, possibly in transit or storage.

Example

The "Instrument and Column Evaluation Calculator" was used to evaluate the variance on an optimized Surveyor HPLC instrument. The efficiency observed was 17% lower than that recorded on the test certificate of the evaluation column (Hypersil GOLD 3µm 150x4.6mm) and this is attributed to the higher variance of this instrument (0.8 σ²) compared with manufacturer's instrument (0.1σ²).

Having evaluated the instrument, we then evaluated a new column (Hypersil GOLD 5µm 50x4.6) by reproducing the test certificate method and comparing the generated efficiency value (86908 N/m) with the expected efficiency value (85816 N/m). The similarity of these values indicated that the column demonstrated similar performance to that at manufacture. The calculator predicted an 11% loss in chromatographic efficiency on this instrument, compared with an actual 10% loss.

The contribution of instruments with larger dead volumes, to total variance, is far more significant on columns such as those packed with 3µm particles (17% loss in efficiency), than, for example, on a similar 5µm column (10% loss in efficiency).

Had an instrument with a variance of 1.4σ² been used then we would expect 20-27% lower efficiency values compared with the values on the test certificate.

Figure 3: Instrument and Column Evaluation Calculator

Instrument and Column Evaluation Calculator
Enter information into the yellow cell ONLY!

Part 1 - Instrument Evaluation
Use a Hypersil GOLD 150x4.6mm 3µm column

Part number of special system evaluation column: 25003-054630

Value	Certificate	Observed
Theoretical plates per metre (half height)	128529	108296
Retention time (min)	2.5	2.5

The estimated variance of your system is Optimization Acceptable 0.8

Part 2 - New Column Evaluation
Use this part to test any new column on the instrument which was evaluated above

Part number of evaluation column: 25009-054630

Value	Certificate	Actual	Expected
Theoretical plates per metre (half height)	86978	86908	85816
Retention time (min)	2.5	2.5	2.5

Part 3 - Results for the New Column Evaluation

The column is showing expected efficiency - any small differences between observed values and those on the test certificate are due to instrument dispersion

Conclusions

- Efficiency is used by chromatographers and manufacturers to determine column suitability and it is important that instrument configuration is taken into account when comparing the performance of a column on different instruments.
- Instrument variance is the sum of the variances produced at different points in the chromatographic system, including the injector, connective tubing and the detector.
- Once an instrument's variance has been established, this can then be used to calculate the expected performance of another Thermo Scientific column on that instrument.
- If the efficiency of a new column, as measured by the chromatographer, is much less than the calculated value, then the column may have been damaged in shipment or storage.
- The "Instrument and Column Evaluation Calculator" can help a determine the variance of an instrument and the suitability of columns tested on that instrument - this is available on request.

Additional Information

For additional information, please visit our Chromatography Resource Centre at www.thermo.com/columns

For a copy of the "Instrument and Column Evaluation" calculator, please contact us at: techsupport.column@thermofisher.com