Measuring and Optimizing Instrument Dispersion

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New Particle Innovations: Ideal for High Throughput*

HPLC separations in one minute or less have become common


Acquity BEH is trademark of Waters Corp.;
Ascentis is a trademark of Sigma-Aldrich.
Origins of Column Band Spreading* (van Deemter)

\[ H = A d_p + B D_m / u + C d_p^2 u / D_m \]

- **A term**: Multipath; bed uniformity; eddy diffusion
- **B term**: Longitudinal diffusion (axial in nature)
- **C term**: Rate of mass transfer from moving phase though stagnant mobile phase into stationary phase (radial in nature)

Instrument impact on \( H \) and \( N \) is often neglected
Peak Band Spreading Equation

\[
\sigma^2_{\text{sys}} = \sigma^2_{\text{col}} + \sigma^2_{\text{instr}} \quad \sigma_{\text{sys}} = \left(\sigma^2_{\text{col}} + \sigma^2_{\text{instr}}\right)^{1/2}
\]

- Band spreading (dispersion) can occur in both HPLC columns and instruments leading to a \textit{system equation}; instrument dispersion is also referred to as instrument bandwidth (IBW)
- New developments in particles have greatly reduced column dispersion; instrument brand, model and configuration now matters; \textit{system} performance can be dramatically different for column and instrument combinations; \textit{can’t use a column without an instrument}!
- New instruments with higher pressure ratings and smaller volume tubing and components have been designed for modern, smaller particle columns; however, traditional instruments may also be suitable for modern columns, especially when optimized by the user; simple tests can qualify instruments for minimum dispersion.
Band Spreading Inside the Column Bed

\[ \sigma_{\text{col}}^2 = V_0^2 (1 + k)^2 / N \]

- \( V_0 \) = mobile phase column volume (\( \mu \)L)
  (unretained peak retention volume; void volume)
- \( k \) = peak capacity factor
- \( N \) = number of column theoretical plates

- Small bed geometry, short retention and high efficiency favor low dispersion (dilution) within a packed column.
- Instrument bandwidth becomes more harmful to efficiency and resolution for short, small ID columns with low \( k \) values and high \( N \).
Illustration of Instrument Dispersion

A peak from the same column in three different instruments.

1. Peak 1 shows a low dispersion instrument where most of the spreading occurs inside the column.
2. Peak 2 shows moderate instrument dispersion in blue.
3. Peak 3 shows high instrument dispersion; peak width has doubled and column peak capacity is halved.

Time spent outside the column destroys system efficiency.
Column vs System Band-Spreading

The effect of system band width can be calculated from the additive relationship of variances, where the total variance of the peak is equal to the sum of the true on-column peak variance plus the instrument variance.

\[
\sigma^2_{\text{system}} = \sigma^2_{\text{column}} + \sigma^2_{\text{instrument}}
\]

\[
\sigma^2_{\text{instrument}} = \sigma^2_{\text{injector}} + \sigma^2_{\text{detector}} + \sigma^2_{\text{connector tubing}}
\]

\[
\sigma_{\text{system}} = \text{Total peak dispersion in volume units (µL)}
\]

\[
\sigma_{\text{system}} = \frac{W_b}{4}
\]

\[W_b = 4\sigma\] is an easy way to estimate dispersion
Direct Method for Measuring IBW

- Connect injector to detector
  - ZDV union
  - shunt
- Inject small volume (μL or less) of chromophore
- Record peak (or retention time and N)
- Calculate IBW (flow in μL) or measure directly from peak retention
  - $\sigma = \frac{(tr \times flow)}{\sqrt{N}}$
  - IBW = 4$\sigma$
- Common mistakes
  - data sampling rate too slow
  - detector response time too slow
  - flow rate too fast (or variable)
  - calculation of N
Comparison of IBW Before and After Instrument Optimization

- Remove column and measure bandwidth at base for small volume injection
- Clear contrast between peak profiles before and after optimization

Shimadzu LC-10A (400 bar) @ flow of 100 µL/min

Before - suitable for 4.6mm columns (30-50 µL)

After - suitable for most columns (15-30 µL)
Test Mix Chromatogram on Ascentis Express Fused-Core C18*

Express peak widths (bands) at base are less than 50µL

Instrument peak width should be smaller, but 50 is about typical for a traditional instrument.

Sample: Uracil, benzene, toluene and anthracene
Column: Ascentis Express C18, 100x4.6mm
Mobile phase: 30/70 water/ACN; Flow: 1.25 mL/min; T = 35°C
Instrument: Waters Acquity, 220 bar (3200 psi)

Fused-Core Measured Plate Height for Unmodified and Modified Instrument

<table>
<thead>
<tr>
<th></th>
<th>Unmodified</th>
<th>Modified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inj</td>
<td>&lt;1</td>
<td>1</td>
</tr>
<tr>
<td>Col</td>
<td>30</td>
<td>72</td>
</tr>
<tr>
<td>Conn Tubes</td>
<td>66</td>
<td>8</td>
</tr>
<tr>
<td>Det*</td>
<td>4</td>
<td>19</td>
</tr>
</tbody>
</table>

* detector cell had a welded heat exchanger that could not be conveniently replaced (Waters Alliance).
HPLC System Components that Affect Measured Peak Bandwidth*

Should also include column selection or bypass valves. Dispersion in injector or other valves and inlet tubing can be reduced by using weak solvent injection or gradients.

Can't remove dispersion in column fittings and frits so it must be treated as an integral part of the column bed.

Dispersion is always a problem after the column.

Electronic and often neglected

Dispersion in Open Cylindrical Flow Path

Time outside column bed is much worse than time inside

\[ \sigma_{\text{tube}}^2 = 1.36 \times 10^{-3} d_t^4 L_t F/D \]

- Dispersion from volume elements is constant for any given flow rate and analyte, but note that dispersion (bandwidth) increases with flow.
- Velocity at the wall is essentially zero under laminar flow conditions. Small inside diameter, short length, low flow and fast solute diffusion favor low dispersion in connection tubes and accessories. Larger molecules show greater dispersion (as 1/D) in connectors.
Performance of a Factory Agilent 1100 Instrument with Sub-2µm Zirconia

A family of curves with $H_{\text{min}}$ ranging from <5µm to >12µm indicates strong instrument impact on $H_{\text{system}}$.

![Plate Height Vs. Linear Velocity for a PBD Column](image)

Plate height vs linear velocity, Temperature 30 °C, Mobile phase: 50/50 ACN/water, Column: 50 x 4.6mm, Agilent 1100/UV with Standard Cell and 0.007” i.d. tubing.
Optimizing Factory Agilent 1100 by Adding Micro Flow Cell

New family of curves with $H_{\text{min}}$ ranging from <5µm to 8µm indicates lower instrument impact on $H_{\text{system}}$

When instrument dispersion is reduced, curves begin to overlap.
Optimized Factory Instrument with Micro Cell + 0.005” ID Tubing

New family of curves with $H_{\text{min}}$ ranging from 4µm to 6µm indicates very low instrument impact on $H_{\text{system}}$.

Instrument dispersion is almost negligible; curves nearly overlap and become flat.

1.6 mL/min test conditions

Plate Height Vs. Linear Velocity for a PBD Column

- Benzene
- Toluene
- Ethylbenzene
- Propylbenzene
- Butylbenzene
HPLC system optimization - tubing

- High Performance Interconnects
- Up 1000 bar
Detector Response can Limit Performance

Column: 50 x 4.6mm C18, 3µm
A: 5% ACN / B: 40% ACN / 0.1%TFA;
Grad: 5.0%B to 100% B in 4.5min
2.5mL/min; 220nm; 30°C

These simple tests should always be run early in a method.
Detector Sampling Rate

Low Sampling Rate

High Sampling Rate
Summary of Variables that Affect Instrument Bandwidth and System Suitability

- Instrument volume should be small with respect to column internal volume which determines peak volume at base.
  - Reduce tubing ID and volume
  - Reduce tubing length and volume
  - Reduce sample volume
  - Reduce organic strength of sample to re-focus analytes on columns
  - Reduce detector flow cell volume
  - Improve detector response time
  - Match data collection rate to peak width in time
    - At least 15 points across the peak
Dispersion References


Dziękuję za uwagę!